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Institute of Physiological Botany
of the University of Uppsala
Box 540
S-751 21 Uppsala (Sweden)

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SUMMARY

Stomatal behaviour in Triticum aestivum L. at the two-leaf stage was studied. The plants were grown either in continuous light or in a day-night light regime and with nutrient media in a concentration series; in addition the concentrations of potassium and nitrate were varied separately. Transpiration was measured with a corona hygrometer or with a thermocouple psychrometer, relative rates of sap flux in the stem were continuously estimated with a thermal method which is briefly described, and changes in stem diameter were measured with a strain gauge transducer.

The plants were tested for cycling tendency (tendency for cycling stomatal behaviour). Cycling in the light was induced by dark pulses or step changes in light intensity or transpiration demand. It was favoured by a high transpiration demand. A lowering of the demand by increasing air humidity or decreasing air flow rate diminished stomatal cycling, and removal of the root resistance by root cutting under water terminated or prevented it. A slowing down of the first stomatal opening in the light with repeated short dark pulses diminished the amplitude of oscillations.

The cycling tendency of plants grown in a day-night light regime showed a day-night rhythm with maxima near the ends of the light periods, whereas it was constant in plants grown in continuous light. Plants grown in continuous light in media with a high potassium concentration (2 mM) showed a high cycling tendency almost irrespective of the concentration of other nutrients. The cycling tendency was low in plants grown with a poor potassium supply (0.2 mM). When all macro nutrients were supplied in low concentrations the plants showed fairly high root resistances restricting transpiration in high light intensities.

Plants grown without a potassium supply developed a severe potassium deficiency and they showed a weak dark-light response and no stomatal cycling. Root treatment with potassium chloride resulted in increased stomatal reactivity and cycling could be induced. It is suggested that

potassium might be transported out of the guard cells to the subsidiary cells during stomatal closing movements in the light and transported in the opposite direction during opening movements, i.e. the potassium content of guard and subsidiary cells would be cycling during stomatal cycling. This would explain the high cycling tendency of plants well supplied with potassium. The possible connection between cycling stomata and a cycling content of abscisic acid in the leaves is discussed.

The transpiration and sap flux curves showed parallel changes when plants with the roots removed under water were subjected to light-dark changes, whereas the stem diameter remained almost constant. In intact plants exhibiting stomatal cycling all curves oscillated with stem diameter minima lagging behind sap flux maxima and these in turn lagging behind transpiration maxima. It was concluded that oscillations in the sap flow curve really indicated oscillating sap flow and that the rapid changes in stem diameter in spite of the influence of growth (increasing the diameter) gave valuable information about the balance between transpiration and water uptake.

The root resistance was varied by partial root removal in air. Sustained oscillations in all recorded parameters were more often induced in plants with the nodal roots removed, and the transpiration level during sustained oscillations was intermediate to that in intact plants and plants with nodal roots and one of three seminal roots removed, both kinds of plants usually showing damped oscillations.

Root excision under water (in the light) resulted in a transient increase in water flow, a transient decrease in transpiration, and a permanent increase in stem diameter. Root excision in air resulted in a transient transpiration increase which was larger if the plants were well supplied with water before excision. Treatment of the roots with NaCl in a high concentration reversed the sap flow direction. Nevertheless, there was a transient transpiration increase. Transient stomatal movements could be brought to enhance or oppose movements going on before excision in water or in air.

It was concluded that transient stomatal movements in detached or intact wheat plants were of the same nature and passive. It was suggested that sustained oscillations in stomatal aperture would require a moderate root resistance and rapid active stomatal movements facilitated by a good potassium availability to the stomatal apparatus and enlarged by passive opening and closing movements.

INTRODUCTION

Stomatal cycling, i.e. rhythmical stomatal opening and closing movements with a period less than 3 hours, has been observed in many plant species, e.g. sunflower (Cox 1968), cotton (Ehrler et al. 1965, Barrs and Klepper 1968), bean (Hopmans 1971), wheat (Rufelt 1956), maize (Raschke 1965, Apel 1967), banana (Brun 1961). Two kinds of cycling have been distinguished, at least in maize. One is CO_2-based with short periods (5 min) (Raschke 1965, Apel 1967), the other is water-based with longer periods (usually 10-50 min) and it is this kind of cycling which is usually observed.

Stomatal movements may be directly observed by microscopic examination of the leaves or of surface impressions of leaves. Indirect methods are more convenient as measurements of transpiration rate, leaf diffusivity, net photosynthetic rate, and leaf temperature. It is agreed that changes in these parameters usually are directly related to changes of stomatal apertures. Changes in other parameters which are more indirectly related to changes of stomatal apertures, have also been used for observing cyclic stomatal behaviour. Such parameters are leaf water status, rate of water uptake, rate of sap flow and stem diameter. During cycling they lag somewhat behind the parameters directly related to stomatal apertures (for a review see Barrs 1971).

Stomatal oscillations may be induced by environmental changes causing aperture changes (see Barrs 1971). The stomata are considered to be incorporated in a feed-back system including negative and positive feed-back loops. Positive feed-back would be a prerequisite for sustained oscillations. Water-based oscillations in stomatal aperture are related to a delay: aperture changes following changes in leaf water potential. Oscillations seem to be favoured by a moderate plant water deficit and a resistance to water uptake or water flow within the plant (Barrs 1971). It is believed that passive stomatal movements (e.g. Raschke 1970) are involved (positive feed-back). Several models of the feed-back system have been worked out (e.g. Karmanov et al. 1966, Lang et al. 1969, Raschke 1970, Hopmans 1971, Cowan 1972).

Florell and Rufelt (1960) reported that temperature and light intensity during cultivation of wheat influenced the tendency of the plants to exhibit stomatal oscillations, a low temperature to a certain extent favouring oscillations. Similar results were obtained for bean by Hopmans (1971), who also found that aeration of the root medium during cultivation lowered the tendency for cyclic behaviour of the stomata.

In the first part of this work the pattern of stomatal behaviour in wheat after different cultivation and experimental treatments was investigated using transpiration measurements. The aim was to find conditions favouring the origin of stomatal oscillations.

In the second part an apparatus for measuring relative water flow rates in the stem was constructed according to Karmanov and Ryabova (1968). The relations between transpiration, sap flow rate, and stem diameter were studied in intact as well as in detached plants. The influence of different root resistances was investigated and also the possible passive nature of transient changes in stomatal apertures.

Part I. Stomatal behaviour in wheat as affected by cultivation pretreatments and experimental conditions.

MATERIAL AND METHODS

Seeds of winter wheat, Triticum aestivum L. cv. Starke (Weibull), were soaked in deionized water overnight and germinated for two days at 25°C in darkness on moist filter paper in Petri dishes. Then the seedlings were placed in perforations made in the cover of 2 l plastic containers supplied with 1.8-1.9 l nutrient medium. 35 seedlings were grown in each container. Several media were used for the cultivation (Tables 1 and 2). When referring to the nutrient medium used the plants will be called A-plants, B-plants, etc. The containers were placed in one of two growth chambers, here in called chambers I and II, and the nutrient media were continuously aerated. During the first part of the investigation the nutrient media were not renewed provided the plants were to be used within 8 days. In other cases the media were renewed after 7 days. Later on a renewal was made after 4 days in chamber I and after 7 days in chamber II.

Chamber I was continuously illuminated from above by fluorescent tubes (Sylvania F 96 T 12-GRO-WHO-L) giving a light intensity of 4-5 klux at plant level. The temperature regulation of the chamber was poor. Air at a temperature of 18-20°C was blown onto the plants but heat emitted from the fluorescent tubes resulted in the temperature of the root medium being up to 6°C above ambient. The relative humidity of the chamber as a rule fluctuated between 55 and 70 %.

In chamber II light and dark periods were 16 and 8 hours respectively. Over the plants were mounted five fluorescent tubes: three Philips TL 40 W/55, one Philips TL 40 W/32 DE LUXE and one Philips TLA 40 W/05. Light intensity at plant level fluctuated between 0.9 and 1.6 klux depending on the age of the tubes. The air was circulated by a fan. The air temperature was 17-19°C. The air humidity was not controlled.

Experiments were carried out using plants with two leaves which were of equal or approximately equal length. This stage was usually reached after 6-8 days in chamber I and after 10-14 days in chamber II.

The plants were transferred to the laboratory immediately before the experiments. 1-8 shoots were inserted in a rectangular perspex cuvette through the open lower end and with the base left outside. The cuvette was sealed with putty (Terostat IX). The roots were immersed in a liquid medium (distilled water unless stated otherwise) which was aerated and, except in early experiments, thermostatically controlled. Outdoor air was filtered (Munktell's hydrofobic air sterilizing filter paper No. 3-A-1) and passed for humidity conditioning (Andersson et al. 1954) through one of five plastic tubes with a length of 1.2 m and an inner diameter of 2.5 cm and half filled with sulfuric acid of appropriate concentrations. The air was blown upwards through the cuvette, filtered again, and led to a humidity sensor. For the purpose of calibration the air was led directly from the tube containing sulfuric acid to the second filter. The inner dimensions of the commonly used cuvette were 200 x 60 x 6 mm giving an air velocity of 7.7 $cm \cdot s^{-1}$ at a flow rate of 100 $l \cdot h^{-1}$. Cuvettes with somewhat different dimensions were also used. This affected the air velocity but did not seem to influence the results.

The plants were illuminated with one or two incandescent lamps (Philips Attralux Spot 24 V 150 W type 13011 E/99) through a 7.5 cm thick water filter and, sometimes, through frosted glass. The light intensity was measured in front of the cuvette with an EEL Lightmaster Photometer.

The air humidity was measured with a corona hygrometer (Andersson et al. 1954, Andersson and Hertz 1955) connected to a recorder. This instrument is sensitive not only to water vapour but also to carbon dioxide. Therefore outdoor air was used to minimize the changes in CO_2 content. Especially during long term experiments there could be some drift in the calibration. On the other hand the instrument had a very short time constant and worked well down to 13 % relative humidity, the lowest humidity used.

The air humidities given by the sulfuric acid solutions were checked with a ventilated psychrometer (Ultrakust Hygrophil, type 4451 A) which was available only during the later part of the investigation. The instrument did not work well at an air flow rate below 100 $l \cdot h^{-1}$. This means that there may be some uncertainty about the humidity values when experiments were performed at lower flow rates. Anyhow, it is a question

of small deviations which will not be discussed further. Sometimes the air flow rate was changed during experiments. This had a slight effect on the sensitivity of the corona hygrometer and hence the calibration. The transpiration was represented by the humidity changes of the air leaving the cuvette (Andersson et al. 1954). Where there may be some doubt about the humidity values these are given within quotation marks (early experiments) or are omitted.

It should be pointed out that the investigation was entirely qualitative. Stomatal behaviour in connection with plant treatments was studied but not quantitative water relations.

Root severing was carried out with scissors. This procedure turned out to be rapid and reliable and no harmful effects of root squeezing during cutting could be detected.

A tendency for cyclic behaviour of the stomata (Barrs and Klepper 1968) will herein be called cycling tendency. It will be related to the extent, the duration, and the amplitude to which stomatal cycling may be induced

RESULTS

A. Preliminary experiments.

Plants grown in media A-K were taken from both growth chambers and tested for their cycling tendency. Parameters varied were the number of plants in the cuvette, the air humidity and flow rate, and the intensity of the illumination given after an initial dark period. Several hundreds of experiments were performed showing that plants cultivated in all the ways mentioned here could be forced to exhibit stomatal cycling under suitable conditions. In most cases damped oscillations appeared when the light was switched on (Figure 3) but sustained oscillations also occurred and sometimes there were no oscillations at all (Figure 1).

Varying results were achieved with plants from chamber II. This was true even when the plants had been grown in the same medium and tested under the same experimental conditions. As an example Figure 2 shows a great difference in stomatal behaviour between plants taken the same day from chamber II at the beginning and at the end of the light period. The

cycling tendency sometimes seemed to be at minimum during the middle of this period (not shown) but as a rule it increased towards the end of the period (cf. Barrs and Klepper 1968).

On the other hand, experiments with plants grown in the continuously illuminated chamber I gave remarkably reproducible results. Figure 3 shows the transpiration curves obtained from four experiments carried out during one day. All curves are of the same shape even though the initial dark period was varied between 5 and 90 min. The closing movement of the stomata was almost finished after 5 min in the dark and the length of the dark period did not seem to be critical between the limits 5 and 90 min insofar as the shape of the curves is concerned. However, a long dark period might give rise to a delay in the stomatal opening after the onset of illumination (Virgin 1956). As a rule the initial dark period was about a quarter of an hour throughout the investigation, the limits being 13 and 30 min. It should also be pointed out that stomatal closure had probably already started when the plants were taken from the growth chamber because the illumination was weak during transport and mounting in the cuvette.

The greatest cycling tendency was often shown by plants cultivated in chamber II and taken for experiments not too early in the light period (Figures 1 A and 2 B). Methods of inducing or inhibiting stomatal cycling were often tested with such plants as well as plants from chamber I. This paper presents some results of these tests achieved with plants cultivated in different media. It should be kept in mind that the wheat plants, irrespective of the cultivation conditions, usually showed the same general pattern of behaviour. However, when the experiments required plants with reasonably constant properties such plants had to be grown in only one medium in chamber I.

B. Effects of experimental treatments.

Dark pulses. Many investigators induced stomatal cycling by giving a dark pulse of appropriate duration as 20 min (Barrs and Klepper 1968), 4 min (Howe 1964), 1-10 min (Hopmans 1971). Nikolič (1925) observed in the microscope a considerable closing movement of the stomata of Zebrina pendula after a dark pulse of only 5 sec. The shortest pulse

tested in the present investigation was 10 sec (one single experiment) giving one trough followed by one peak in the transpiration curve (not shown). Dark pulses of 1-3 min were often used and Figure 4 shows a sustained oscillation induced in this way. However, in many cases the oscillation induced was damped giving only a few peaks and troughs.

Light intensity changes. Figure 5 shows the effects of light intensity changes, probably connected with leaf temperature changes (not measured) which might cause transpiration changes even without changes in stomatal aperture. However, the overshoots in the curve indicate that stomatal movements really occurred. Moreover, stomatal cycling was induced by the decrease in the light intensity.

Changes in transpiration demand. The transpiration demand in the cuvette could be increased by increasing the air flow rate or by decreasing the air humidity, the opposite changes resulting in a decreased transpiration demand. Results of sudden changes in the air flow rate are shown in Figure 6. The corona hygrometer is mainly sensitive to the absolute humidity (Andersson and Hertz 1955). This means that the curve parts a-b, c-d, and e-f represent the instantaneous changes in the air humidity which owing to the water transpired occurred in the cuvette when the flow rate was altered (disregarding the small changes in the calibration, see Material and Methods). Changes in the transpiration rates have to be calculated with regard to the changed air flow rates. For instance, the curve part a-b shows a rapidly increasing air humidity. However, the decreased air flow rate also meant a decreased air velocity probably leading to an increased boundary layer resistance and hence to a lowered transpiration rate (Grace and Thompson 1973, cf. also Stålfelt 1932). The increased air humidity would have worked in the same direction. Thus, point b represents a higher air humidity but probably a lower transpiration rate than point a. On the other hand, the curve parts b-c, d-e, and f-g represent transpiration changes caused by stomatal movements. That this is so, can be seen from Figure 7. In this experiment the air flow rate and the light intensity were changed simultaneously so that the changes were expected to oppose each other with regard to their effects on the stomata. The curve parts a-b and c-d represent the same kind of rapid changes in the air humidity

as those shown in the corresponding curve parts of Figure 6. But the following slower changes were in the opposite direction to those shown in Figure 6, i.e. changes in light intensity predominated over changes in air flow rate in the experiment illustrated by Figure 7. From the two experiments (Figures 6 and 7) it was concluded that stomatal movements were induced by changes in the air flow rates. A decrease led to a further stomatal opening and more or less inhibited stomatal cycling and an increase led to a partial stomatal closure and seemed to induce cycling.

Figure 8 shows two experiments, one of them starting with humid and the other with dry air. The plants exhibited a much higher cycling tendency in dry than in humid air and a short dark pulse induced cycling only in the plants in dry air. Humidity changes at constant flow rate led to the same kind of response as humidity changes caused by air flow rate changes (Figure 6). The cuvette absorbed water causing delays in the registration; perspex is not an ideal material for cuvettes.

Root cutting under water. Cutting of roots or stems in the root medium is known to stop oscillations or to prevent them (e.g. Barrs and Klepper 1968, Brogårdh and Johnsson 1973). The sustained oscillation shown in Figure 1 A was terminated in this way. This was the normal result of root removal under water during stomatal cycling irrespective of the phase of the oscillation.

Severing the roots under water during the initial dark period prevented stomatal cycling. An example is shown in Figure 9 B. The intact plants (Figure 9 A) had a high cycling tendency and opened their stomata quickly to a first maximum which was also the largest one. The average transpiration rate seemed to be about half the first maximum. The rootless plants opened their stomata slowly but the transpiration rate increased to about the same maximal level and then remained constant. This was a typical result; oscillations were connected with a low transpiration rate compared with that of plants having a constant transpiration rate after stomatal opening (see also Figure 1).

Repeated dark pulses. A slowing down of the first stomatal opening in intact plants with many short dark pulses (cf. Virgin 1956) did not

entirely prevent cycling, but decreased the amplitude (Figure 9 C). The average transpiration rate was at first intermediate to those in the two preceding experiments and then slowly decreased.

C. Influence of different nutrient supplies.

Plants growing in the media A-K differed in growth rate and in size and condition at the two-leaf stage. Medium A was the richest one and apparently permitted the best growth. D was the poorest medium giving tiny, pale plants. Damped oscillations in D-plants were induced but D-plants were not used very much in the present investigation because of their bad condition. However, if the D medium was renewed every third day the plants got a better condition and turned out to be useful for certain experiments (see Part II).

The climatic conditions of chamber I favoured rapid growth, whereas the plants in chamber II grew slowly and became rather weak. It could also be a difference in stomatal behaviour between plants grown in the same medium but in different chambers (Figures 2 and 3).

The cycling tendencies of plants grown in different media in chamber II were difficult to compare because of the effects of the day-night rhythm in the light regime. A comparison was made between plants A-C and E-K grown in chamber I. The number of plants in the cuvette was varied in order to compensate for differences in plant size. Only a few experiments were performed with C-, G-, H-, and K-plants. This reservation being made, Figure 10 shows some trends (see also Table 2).

Intense stomatal cycling was induced in plants grown in media with a high potassium and a low nitrate concentration (G, E, and F) almost irrespective of the concentration level (A, B, or C) of other nutrients. Cycling with nearly the same intensity was induced in A-plants supplied with both potassium and nitrate in high concentrations. On the other hand, only a slight cycling was induced in plants grown in media with a low potassium and a high nitrate concentration (H, F, and K). Thus, for instance, the cycling tendencies of E- and F-plants (supplied with other nutrients on the B level) were very different which is also demonstrated in Figure 11. Sometimes even F-plants could exhibit

sustained oscillations (not shown) when exposed to very dry air (13-20 % relative humidity). B-plants might be considered "average plants" with regard to the supply of nutrients as well as to their cycling tendency.

C-plants, grown in the poorest medium tested, opened their stomata to a first overshoot but cycling did not occur. However, sometimes cycling was induced in C-plants from chamber I. When a C-plant was illuminated with a very high light intensity (beyond the scale of the lightmeter), the transpiration stabilized on a low level after a first maximum (Figure 12). Root excision under water resulted after a first further transpiration decrease in an increase far beyond the first maximum indicating that the root resistance had been very high limiting the water supply. No similar decrease in the transpiration was observed in A- and B-plants illuminated in the same way.

Potassium seems to be accumulated in the guard cells during stomatal light opening and transported out of them during dark closing (Fujino 1967, Fischer 1968, for further references see Imamura 1943, Raschke and Fellows 1971, Penny and Bowling 1974). The difference in stomatal behaviour between E- and F-plants suggests the possibility that the amounts of potassium available to the stomatal apparatus in wheat might be related to the cycling tendency. If so, potassium starvation would lead to drastically reduced oscillations. A potassium supply to such plants might restore the cycling tendency (cf. Peaslee and Moss 1966).

Wheat plants were grown in the L medium which was identical with the A medium except that K was exchanged for Na (Table 1). Sugiyama et al. (1968) reported "a drastic decrease in the content of K^+" in the first leaves of wheat plants cultivated without K supply for 12 days in 14°C (the light regime not mentioned). After 7 days in chamber I with its continuous light and a temperature of about 20°C the L-plants were evidently suffering from K deficiency showing necrotic spots on the first leaf.

Plants grown in the L medium for 5-7 days were used for the experiments which were extended overnight. When the root medium was distilled water (Figure 13 A) the transpiration rate was relatively high in the

dark and changed only a little in the light. No oscillations occurred. This indicated that the dark-light response of the stomata was very weak. On the other hand, when the root medium from the beginning of the experiment was a KCl solution (Figure 13 B) the reactivity of the stomata to light changes was increased after some time and a dark pulse then induced a weak cycling. Another example of stomatal recovery is shown in Figure 14. Here the initial dark period was prolonged and the first weak stomatal opening in the light was followed almost immediately by a further slow opening. A first dark pulse after a few hours revealed a somewhat increased stomatal reaction but cycling was not induced. After a second dark pulse given much later, the difference between light and dark transpiration proved to be even larger and sustained cycling was induced. Still, the dark transpiration of KCl-treated L-plants was relatively high. The stomatal reactivity of the L-plants was considerably increased when the root medium during the experiments was a KCl solution with a concentration ranging from 2 to 20 mM. About the same effect was achieved with the A medium.

DISCUSSION

Stomatal cycling may be induced by a sudden change in some factor directly or indirectly affecting the stomatal aperture (Barrs 1971). Oscillations were induced by changes in light intensity, transpiration demand, or both. Increased light intensity (Figure 5) almost always resulted in further stomatal opening and sometimes in cycling. A lowering of the transpiratory demand by manipulating the air flow rate (Figure 6) or air humidity (Figure 8) also resulted in further stomatal opening (cf. Schulze et al. 1972) but cycling was diminished or inhibited as reported for bean by Hopmans (1971). The wheat stomata diminished their apertures when subjected to an increased transpiratory demand (Figures 6 and 8) and similar results were reported for wheat (Heath and Milthorpe 1950) and for Xanthium (Drake et al. 1970). Cycling was often induced. A decreased light intensity also resulted in diminished stomatal aperture, sometimes followed by cycling (Figure 5). This effect was reported for maize by Karvé and Mishal (1966). It is in accordance with the finding that sustained cycling sometimes was

induced by switching on the light to a low intensity. C-plants obviously possessed a high root resistance (Figure 12) and their stomata could be triggered to show cycling mainly in light of lower intensities (Figure 1 A). Thus, stomatal cycling in wheat could be induced by a "photo-active" as well as by a "hydroactive" closing movement (Stålfelt 1955).

As a rule root cutting under water prevented or stopped cycling (Figure 1 A). In experiments extended for several hours oscillations sometimes continued after root removal or could be induced by a dark pulse. Later, regardless of root cutting, the transpiration rate and the turgor decreased, probably owing to the harmful influence of distilled water (Mingeau 1969, Talha and Larsen 1975). Oscillations in rootless plants might be favoured by secondarily induced resistances in the water pathway (cf. Hopmans 1971).

Factors essential for the origin of stomatal cycling in the light in wheat seemed to be the same as those reported for other species and reviewed by Barrs (1971):

a. induction of a sudden change of stomatal aperture no matter in what direction,

b. a not too low transpiratory demand,

c. a resistance to water flow causing a delay in the water supply during stomatal opening.

In the model of stomatal behaviour made by Cowan (1972) oscillations in stomatal conductance were favoured by a high potential transpiration (rate of evaporation if the conductance of leaf to diffusion of vapour were infinite), a high root resistance, and a low base water potential. Cowan pointed out that real plants might behave differently from the model. The behaviour of the C-plants grown in chamber II is one example. A high root resistance should be combined with a relatively low potential transpiration for oscillations to occur. This is in accordance with the finding of Brogårdh et al. (1974) that the magnitudes of root (or plant) resistance, water potential in the root medium, transpiratory demand and light intensity have to be combined within restricted limits for sustained oscillation, other combinations giving damped oscillation

or stable transpiration. Other examples are given in the literature: Sustained stomatal cycling in bean ceased when the light intensity was increased and also when it was decreased (Karmanov et al. 1966). Hopmans (1971) induced cycling in bean by watering and cycling faded away during drying of the "soil" (peat-perlite). Cox (1968) reported that cycling in sunflower ceased when the soil water potential was lowered. Brogårdh et al. (1974) found the same phenomenon for oat after lowering the water potential of the root medium with mannitol.

As the wheat roots were surrounded by an aerated nutrient medium with a fairly high water potential, water stress would be caused by internal resistances. These were mainly located in the root system (Figure 12). As pointed out by Barrs and Klepper (1968) there may be an increase in the resistance to water flow during the light period giving plants with cycling tendencies varying in a day-night rhythm. Hopmans (1971) identified this rhythm as circadian for bean. The wheat plants cultivated in chamber II showed a daily rhythm in the cycling tendency (Figure 2). The reason would probably be the same; a daily rhythm in the resistance and maybe also in the water potential. This rhythm was absent in plants cultivated in continuous light in chamber I (Figure 3). Sometimes the amplitude of oscillations induced in plants taken from chamber I late in the evening was a little lower than that induced in plants taken earlier the same day. It was not investigated whether this was dependent on a circadian rhythm or was simply a result of leaf ageing. However, Parsons and Kramer (1974) reported diurnal cycling in the root resistance of cotton plants grown in a day-night light regime but no cycling occurred when the plants had been grown for 8 days or more in continuous light.

Plants grown with a good potassium and poor nitrate supply (G,E,J) showed a high cycling tendency (Figures 10 and 11 B, Table 2). The role of the low nitrate concentration is difficult to elucidate because it was combined with a high concentration of chloride. However, A-plants grown without chloride and with high potassium and nitrate concentrations showed a fairly high cycling tendency indicating that potassium was the relevant ion with regard to cycling. Media with potassium partially replaced by sodium (H,F,K) gave plants with a low

cycling tendency (Figures 10 and 11 A) and this diminished to zero in the L-plants as potassium deficiency developed in the leaves. Cycling could be induced after treatment of the roots of L-plants overnight with potassium chloride (Figures 13 and 14). The relatively high rate of dark transpiration also after this treatment might be related to necrotic spots on the leaves. Another possibility is that the stomata of L-plants were irreversibly affected by the potassium deficiency. Treatment of C-plants overnight with potassium chloride seems to improve both the dark closure and the cycling tendency (not shown). These experiments are to be continued.

The root permeability to water may be decreased by potassium deficiency (Graham and Ulrich 1972) and increased by a subsequent treatment with a medium containing potassium. Stomatal opening in the light requires influx of potassium to the guard cells (e.g. Humble and Hsiao 1969). These two circumstances may explain the recovery of the L-plants (cf. Peaslee and Moss 1966). The various cycling tendencies of the plants tested in this investigation might be related to various plant resistances to water flow depending on growth conditions (Barrs and Klepper 1968).

It is known for several species that potassium accumulates in the guard cells during stomatal light opening and is transported out of them to the subsidiary cells during dark closure (for maize see Raschke and Fellows 1971). Recently, abscisic acid (ABA) has been shown to be a powerful agent causing stomatal closure (Mittelheuser and van Steveninck 1969, for a review see Milborrow 1974). It is reported that ABA prevents flux of potassium into the guard cells in epidermal strips of Commelina (Mansfield and Jones 1971) and Vicia (Horton and Moran 1972). It might be inferred that stomatal closure in the light initiated by ABA would be related to potassium flux from the guard cells but so far little research has been done on this topic. However, Imamura (1943) found that potassium was accumulated in the guard cells of open stomata of Zebrina pendula and was equally distributed in epidermis with closed stomata irrespective of the cause of closure; darkness, water shortage or even watering (!). Stomatal closure commenced within 5 min when detached barley leaves

were supplied with ABA via their cut ends (Cummins et al. 1971). The stomatal reaction was reversible; stomata opened when ABA was removed from the solution. Cummins et al. concluded: "Rapid production, rapid specific action on stomata, and rapid inactivation or sequestering of ABA may thus be processes involved in a "hydroactive" feed-back loop controlling water loss of a leaf."

The hypothesis is now proposed that stomatal cycling in wheat might be connected with a "shuttle" of potassium between the guard cells and the subsidiary cells (Raschke and Fellows 1971). A low potassium standard in the leaves would be sufficient for stomatal opening in the light but larger amounts of potassium would be required to allow rapid changes of osmotic potential in the guard cells leading to rapid stomatal movements and thus favouring cycling (Figure 10). Chloride as a counter ion might enhance the effect of potassium (cf. Raschke and Fellows 1971). The two cases, the low and the high potassium standard, might be illustrated by the gradual recovery of L-plants supplied with potassium chloride (Figure 14): the first dark pulse did not initiate cycling, the second did, the stomatal movements now being more rapid.

The proposed mechanism demands a rapid and sensitive adjustment of the ABA level lagging somewhat behind the changes in water potential as suggested by Hiron and Wright (1972). Not much is so far known about this. Loveys and Kriedemann (1973) found that the content of ABA-like inhibitors was rapidly enhanced during water stress and stomatal closure in excised vine leaves. On the other hand, Zabadal (1974) questioned whether a small increase in the ABA content could account for a great change in stomatal resistance within 15 min. Further research is necessary on this topic. The possible connection between the ABA level and transport of potassium out of the guard cells in the light also needs to be investigated.

Part II. Relations between transpiration, water transport and stem diameter in wheat before and after root treatment with osmotica or root excision.

MATERIAL AND METHODS

The wheat cultivar Starke was replaced by Starke II (Weibull) for most experiments. The plants were usually cultivated in chamber I as described previously (Part I). However, the D medium was renewed every third day. Chamber I was later provided with improved temperature and humidity controls (set to 20 $\pm$ 1°C and 50-60 % relative humidity, respectively). The fluorescent tubes were replaced by Sylvania F 96 T 12-GRO-WHO-WS tubes giving 3-6 klux at plant level depending on the age of the tubes, usually 4-5 klux. Some plants (Figure 24) were cultivated in a greenhouse with daylight plus continuous light from fluorescent tubes (Osram-L-Fluora 40 W/77).

During the investigation the laboratory was provided with facilities for temperature regulation, first down to 23°C, and later to lower temperatures. Since then 20°C was chosen as standard temperature. The initial dark period was 12 min in one (Figure 24), 40-50 min in two (Figures 17 and 18), and about 30 min in remaining experiments. Root media were rapidly changed with syringes. The temperature difference between the media was held within 0.1°C. Then an exchange of solutions of the same kind did not influence the recording. Sometimes the roots were cut twice, first under water with 1-1.5 cm stumps left, then in air.

Experiments were carried out with A- and D-plants at the two leaf stage. The experimental arrangement is illustrated in Figure 15. One single plant was mounted in a cuvette through a rubber cork. The root medium (distilled water unless stated otherwise) was not aerated, nor was it thermostatically controlled. Dry air was led into the cuvette, the humidities ranging from 10 to 33 %, from 10 to 18 % in the series of experiments depicted in Figures 19-21.

Air humidities were measured with the corona hygrometer at the beginning (Figures 17, 18 and 23), and later with a thermocouple psychrometer with one dry and one wet thermocouple. This was altered several times because of trouble with the water supply. The psychro-

meter worked satisfactorily when provided with a feeding wick (Mitchell et al. 1973).

Changes in stem diameter were measured with a strain gauge rectilinear displacement tranducer (Swema SG 4-25) (cf. Fries et al. 1974).

Relative sap flow rates in the stem were measured with an apparatus constructed according to Karmanov and Ryabova (1968) (Figures 15 and 16). Two thermistors coupled in a bridge were mounted in a perspex holder symmetrically on both sides of a separate heating thermistor. The distance between the outer thermistors was 10 mm. The holder had an U-shaped slit through which a wheat stem was placed. The thermistors were movable against weak springs. The stem was gently pressed against the thermistors with a perspex plate. The bridge was balanced before the experiment. The heating thermistor was supplied with a continuous current adjustable between 1.5 and 15.7 mA giving an effect of approximately 6-60 mW. The sap flux in the stem transported heat to the "downstream" thermistor (T_2 in Figure 16). This changed its resistance and the bridge was out of balance. The imbalance was amplified and the signal recorded. The general shape of the curves obtained were not influenced by the magnitude of current chosen for the heating thermistor, but distortions due to outer influences were diminished when a current towards the upper end of the range was supplied. The first imbalance was recorded within 15 sec after the current was switched on in the dark.

Only relative rates of sap flux were recorded. Karmanov and Ryabova (1968) found that the imbalance of the bridge within certain limits was proportional to the sap flow rate. However, there might be some uncertainty about the true flow rates, and the apparatus is therefore more suitable for estimation of relative flow rates (e.g. Meleshchenko and Karmanov 1974). On the other hand, it is possible to record the direction of the sap flow and thereby changes of direction.

Since changes but not values of sap flux rate and stem diameter were recorded, the time course of all parameters are given as "recorder response" without any calibrations.

Figure 17 shows the relations between transpiration, sap flux, and stem diameter in a plant with the roots removed under water during the initial dark period. The transpiration and sap flux curves parallel each other without a time lag. The stem diameter remained nearly constant. As a contrast Figure 18 shows curves obtained with an intact plant. Stem diameter minima lagged behind sap flux maxima and these in turn lagged behind transpiration maxima. This indicated the importance of the root resistance for the origin of water deficits in wheat plants.

A lag in the water supply behind the transpiration seems to be essential for the origin of stomatal cycling (Ehrler et al. 1965, Lang et al. 1969). The cycling tendency should be expected to increase with increasing root resistance according to the model of stomatal behaviour developed by Cowan (1972). This is not always true for real plants as has been shown for C-plants (Part I).

The total root resistance R of a plant with i roots, the resistances of which are r_1, r_2, ... r_i may be calculated from the formula

$$\frac{1}{R} = \frac{1}{r_1} + \frac{1}{r_2} + ... + \frac{1}{r_i}$$

Removal of some of the roots in air leads to an increased total resistance to water flow of the remaining root system, at least in the very beginning. The plant may partially compensate for the loss of some roots by decreasing the resistance of the remaining roots as a result of increased suction (e.g. Shirazi et al. 1975). However, it seems reasonable to assume that the resulting resistance in every moment be higher than that of an intact root system in accordance with the finding of Passioura (1972) that single-rooted wheat plants had a root mass of the same magnitude as that of normal plants but used less water than these because of increased root resistances.

The wheat plant at the two leaf stage usually had 3 seminal and 2-4 nodal roots. The seminal roots were longer and more branched than the nodal roots. Some of the roots were cut in air during the initial dark period, and the stumps were squeezed to prevent air entrance to the xylem. Plants treated in the same way showed great

variations in response to light, for the transpiration ranging between a few peaks and sustained oscillation. However, Table 3 shows that removal of the nodal roots of A-plants strongly enhanced the probability for sustained oscillation to occur, whereas further root removal decreased this probability again. Figure 19 gives an example of typical behaviour of intact plants. Damped oscillations were induced in all parameters recorded. The transpiration remained on a moderately high level. Figure 21, on the other hand, shows the typical behaviour of plants with only two remining seminal roots. The transpiration decreased to a low level after a few peaks. The sustained oscillation (Figure 20) seemed to represent an intermediary water consumption, the mean level of the transpiration being between that of the two other experiments. It should be pointed out that what is compared is not the absolute transpiration rates but the rates related to the first peaks.

Figure 20 shows the effect of root cutting under water during stomatal cycling. There was a large transient increase in the sap flux (cf. Brogårdh and Johnsson 1973) and simultaneously a permanent increase in the stem diameter. On the other hand, there was a transient decrease in transpiration. The regular oscillations in all parameters ceased.

Many experiments were carried out to study this phenomenon: the transient decrease in transpiration after root excision under water. It was large when the transpiration was on a high level just after a transpiration peak. Root cutting at a transpiration through usually resulted in a smaller decrease. This can be readily explained. The stomata were already partially closed at transpiration minima. Plants subjected to water stress exhibited a relatively large transient transpiration decrease after root cutting. This is illustrated in Figure 20. The plant had no nodal and only two seminal roots; the water uptake was restricted. The transient transpiration decrease was large in spite of root cutting just before a transpiration minimum.

Figure 21 illustrates a more restricted transpiration. This plant also had two remaining roots (seminal). One root was cut under water resulting in the same rapid changes of the three parameters as described above. However, oscillation was induced. This was repeated when also the other root was cut under water but the oscillation soon faded away. Oscillations were rarely induced by root cutting under water particularly not if the cutting was followed by a dark period. However, a dark pulse sometimes induced stomatal cycling in rootless plants. This seems to be the case in the experiment depicted in Figure 17. Other similar experiments revealed that the transpiration and sap flux curves paralleled each other also during cycling.

Falk (1966 a) found for wheat that the transient transpiration increase after stem or root excision in air, the so called Iwanoff effect (Iwanoff 1928), was decreased with increasing light intensity. That was also the case in this investigation. Figure 22 A shows a minimal transient transpiration increase after root cutting in air, when the light intensity was 80 klux. The roots were first cut under water in another experiment carried out at the same high light intensity (Figure 22 B). The transpiration stabilized on a higher level after the transient decrease. Then the transient increase after excision of the root stumps in air was large. The sap flux curves were omitted from the figures because of distortion. However, there was a transient increase after root cutting under water and later the curve stabilized on a higher level than that before cutting (cf. Figure 26). Both sap flux curves showed a steep decrease after root excision in air. All roots except one seminal were cut in air during the initial dark period. This restricted the water supply when the light was switched on. The transpiration curves (before further root cutting) resemble that depicted in Figure 21. However, the larger decreases in stem diameters (Figure 22) would be due to larger water deficits (see the discussion). An extreme example of this is shown in Figure 26.

A sudden treatment of the roots on intact plants with osmotica during the light period also results in a transient transpiration increase (e.g. Rufelt 1956, Falk 1966 b). Falk recorded the water uptake using a potometer. At the moment of medium exchange there was a "silent

minute" in the recording (in reality about half a minute). Falk concluded from the form of the curve parts that the transient transpiration increase after a mannitol supply was connected with a transient decrease in water uptake (which could not be recorded).

A series of experiments (not shown) with mannitol revealed that Falk was quite right. A rapid transient decrease of the sap flow rate preceded the transient increase in transpiration rate. A more extreme experiment is shown in Figure 23. The water was here replaced by about half concentrated NaCl. The effect was violent. The large transient decrease in the sap flow curve indicates that the flow direction was reversed for a short while and water was drawn out of the roots. The rapid decrease of the stem diameter is in accordance with this assumption. Nevertheless, there was a transient increase in the transpiration.

However, an experimental difficulty was demonstrated in this experiment. The sap was warmed up when passing the heating thermistor and the upper ("downstream") thermistor (thermistor 2, Figure 16) was warmed up in turn, lowering its resistance, thus giving an imbalance to the bridge. At the beginning of a reversed sap flow there would be an aftereffect of the increased sap temperature leading to a smaller imbalance of the bridge (with opposite sign) than what would be expected. The result might be that a reversal of the flow direction will not be detected if the duration of the reversed flow is very short. Figure 24 shows a possibility to overcome this difficulty. Concentrated NaCl was used in this experiment. In fact, that was not a good idea; the author feels that somewhat diluted solutions are more suitable for this kind of experiments. The current through the heating thermistor was zero from the start of the experiment. The water was removed with a syringe. The current to the heating thermistor was switched on a few seconds before the addition of NaCl. The steep decrease in the recorder response reveals that a reversal of the sap flow direction really occurred. There also was a transient transpiration increase.

Some experiments were carried out to investigate whether the transient stomatal movements just described might influence "photoactive" opening and "hydroactive" or "photoactive" closing movements (Stålfelt 1955). Figure 25 shows some results of these experiments. The stomatal

opening in light was changed to a transient closing movement when the roots were cut under water (Figure 25 A). On the other hand, the same treatment during "hydroactive" closure increased the rapidity of the stomatal movement: the curve became steeper (Figure 25 B). Both experiments were continued with excision of the root stumps in air. However, the light was first switched off and the excisions were not undertaken until the "photoactive" closure had started. This stomatal movement was also changed to a movement in the opposite direction resulting in a transient transpiration increase.

The effect of root cutting under water during more pronounced water stress is shown in Figure 26. A D-plant with all roots but one seminal removed in air was subjected to light of high intensity. The transpiration curve resembles that of the stressed C-plant (Figure 12, Part I). However, the result of root cutting under water was different. There was a transient transpiration increase followed by a transient decrease after which the transpiration increased to a fairly high level. The transient increase in flow rate was tremendous. The large "Iwanoff effect" at the end of the experiment demonstrated that the water stress was overcome.

This sequence of stomatal movements was also achieved after root treatment with a diluted solution of sodium-diethyl-dithio-carbamate (DIECA) followed by excision under water, demonstrated earlier by Rufelt (1956). Treatment of the roots with 10^{-5} M $AgNO_3$ or ice water before root excision resulted in the same phenomenon.

DISCUSSION

A comparison between Figures 17 and 18 gives a possibility to elucidate for what purpose the flux meter built according to Karmanov and Ryabova (1968) may be used. The flux curve in Figure 17 follows the transpiration curve closely while the stem diameter curve, at least for the first light period, is nearly horizontal. This implies that the signal from the flux meter was more or less directly proportional to the sap flux rate provided that the transpiration curve reflected the transpiration rate cor-

rectly. It would be possible to calibrate the flux meter for the case that there is very little resistance to water uptake, i.e. the stem diameter is constant. However, the output from the bridge might vary with the stem diameter for anatomical reasons (cf. Sheriff 1974) and moreover, it might vary with the application of the thermistors to the stem. This would make necessary a calibration for every plant.

The situation is even more complicated in the case of oscillating stem diameter. It may be argued that the signal from the flux meter is proportional not only to the flow rate but also to the velocity of the sap flow. Then the signal might oscillate under constant flow rate provided that the diameter of the conducting vessels oscillates (cf. Karmanov et al. 1969). This may be true to a certain extent. However, the three curves of Figure 18 lag behind each other, the phase angle between transpiration and flux maxima and between flux maxima and diameter minima being about $\frac{\pi}{4}$. That implies that the sap flux really oscillated. However, it is so far not possible to state strict proportionality between the flux and the flux curve. It is to be investigated whether a reliable calibration is possible.

The transient changes of flow rate and flow direction are probably impossible to calibrate. However, the instrument turned out to be a suitable means to detect those transients.

It is also questionable whether the stem diameter of wheat was related to the water potential by a single-valued function. Klepper et al. (1971) found a daily hysteresis between stem diameter and water potential in cotton, and Molz and Klepper (1973) concluded from this and from the slow changes in stem diameter after watering or severing the roots under water that it would not "be possible to develop a simple relationship between cotton stem diameter and plant water status such that a measurement of stem shrinkage could be used to estimate plant water potential directly". On the other hand, the herbaceous wheat stem changed its diameter rapidly after rapid changes in the sap flow rate or transpiration rate. Thus, it seems reasonable to assume that changes of the stem diameter of wheat, at least to a certain extent, reflected quantitative differences between transpiration and water uptake. However, it should be kept in mind that growth increased

the stem diameter. This interfered with the changes caused by other factors.

The root resistance seems to be critical for the occurrence of sustained oscillations (Figures 19-21, Table 3). The A-plants illuminated with a moderately high light intensity evidently were able to transpire on a high level, the oscillations induced usually fading out. However, a moderate increase of the root resistance after removal of the short nodal roots was sufficient in many cases to lower the mean transpiration rate and permit sustained oscillations in all recorded parameters. Further root removal led to damped oscillation and still lower transpiration rate. These results are in agreement with those of Brogårdh et al. (1974), who terminated oscillations by lowering the water potential of the root medium, and they support the view that the model of Cowan (1972) needs some alterations to account for the cessation of oscillations when the water potential of the root medium was lowered or the root resistance was increased beyond certain limits (see Discussion in Part I). Such improvements were suggested by Brogårdh et al. (1974).

The transient transpiration changes after root or stem excision or after treatment of intact plants with osmotica or temperature changes are often regarded as caused by passive stomatal movements: stomatal opening following upon a sudden water shortage resulting in rapidly decreasing turgor in the subsidiary cells, stomatal closure following upon a sudden increase in the water supply resulting in rapidly increasing turgor in the subsidiary cells (e.g. Darwin and Pertz 1911, Heath 1938, Monzi 1939, Raschke 1970).

Transient stomatal movements have also been regarded as active. Tension release after excision in air (Renner 1929, Iwanoff 1928, Andersson et al. 1954, Decker and Wien 1960) or increased root permeability after root treatment with osmotica (Rufelt 1963) would cause a transient increase in the water supply to the guard cells giving stomatal opening, and excision under water would remove the root pressure giving a transient decrease in the water supply to the guard cells and hence stomatal closure (Andersson et al. 1954).

The results of this investigation support the view that transient transpiration changes are related to passive stomatal movements

(Figures 20-24). According to a mechanical analysis carried out by DeMichele and Sharpe (1973) the subsidiary cells would have a mechanical advantage over the guard cells, the stomatal aperture being the result of opposing pressures of the guard and subsidiary cells (see also Heath 1959). Equal reductions in turgor would result in increased aperture (passive opening) until the subsidiary cells become flaccid. At that moment the aperture would be at maximum. It may be argued that equal increases in turgor of the guard and subsidiary cells for the same reason would result in a (passive) reduction of stomatal aperture.

It was almost impossible to add two temporary transpiration increases. The transpiration was not influenced by root cutting in air just at the top of a temporary transpiration increase achieved after treatment of the intact plant with osmotica (see also Falk 1966 b) or ice water. Karmanov et al. (1966) reported a transient increase in sap flux and a transient decrease in transpiration rate (cf. Figure 20) after watering intact plants. Gee et al. (1973) applied pressure to roots of intact plants also resulting in a transient transpiration decrease. These results suggest that transient transpiration changes in intact plants are of the same kind as those in detached plants. This is to be expected according to the analysis by DeMichele and Sharpe (1973).

The stomatal behaviour illustrated in Figure 26 was observed by Monzi (1939). He regarded the transient stomatal closure as passive. That conclusion seems to be correct. The course would be this: The turgor in the guard as well as in the subsidiary cells would be very low just before root excision. Both kinds of cells receive water after the excision and stomatal opening commences. Still, the turgor of the subsidiary cells is too low for the cells to oppose stomatal opening. However, they rapidly regain turgor, and there will be a temporary passive stomatal closure of the same kind as described above.

If the transient stomatal movements are passive, they would be able to interfere with active movements. From Figure 25 it can be seen that this was the case. The transient movements opposed effectively the active for a short while. On the other hand, a transient movement operating in the same direction as an active enhanced the effect of this (Figure 25 B).

Passive stomatal movements are believed to be involved in cycling in a positive feed-back loop (Barrs 1971). The result of root excision in air at the first large transpiration maximum was either a minimal or no transient increase in the transpiration. This indicated a low turgor of the subsidiary cells before excision, i.e. the large transpiration maximum was partially due to passive opening. A low water deficit before a sudden onset of water stress favoured a large passive opening (Figure 22) as also reported for cotton (Brittain and Nagarajah 1971). On the other hand, a moderately large water stress before excision favoured a large passive closing movement after a rapidly enhanced water supply (Figures 20 and 22 B).

In plants with a low root resistance and a sufficient water supply passive stomatal opening might contribute little to the positive feed-back loop leading to increased stability of the stomatal control system: oscillations would fade out (Figure 19). In plants with a high root resistance and a restricted water supply the contribution to the positive feed-back loop from passive closing movements would be little with similar consequences: oscillations would fade out (Figures 21 and 22). In plants with a moderately high root resistance both passive opening and passive closing movements would occur leading to a high instability of the stomatal control system and sustained oscillations (Figure 20). Rapid active stomatal movements (discussed in Part I) would be a prerequisite for the origin of passive movements in intact plants.

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Table 1. Composition of nutrient media. Media A-D constitute a series of concentrations. Thus B = 0.325 x A, C = 0.10 x A, D = 0.0325 x A. No microelements were added. Deionized water was used. During the first time Fe was given as a mixture of $Fe_2(SO_4)_3$ and EDTA.

Medium	Concentration, mM									
	KCl	KNO_3	$NaNO_3$	$Ca(NO_3)_2$	$CaCl_2$	KH_2PO_4	NaH_2PO_4	Na_2HPO_4	$MgSO_4$	Fe-EDTA
A		1.00		1.00		0.95		0.050	0.50	0.040
B		0.330		0.33		0.31		0.016	0.16	0.013
C		0.100		0.10		0.095		0.0050	0.050	0.0040
D		0.033		0.033		0.031		0.0016	0.016	0.0013
E	1.69			0.15	0.18	0.31		0.016	0.16	0.013
F			2.35	0.33		0.20	0.11	0.016	0.16	0.013
G	0.70	0.300			1.00	0.95		0.050	0.50	0.040
H		0.200	0.80	1.00			0.95	0.050	0.50	0.040
J	1.60	0.300			0.10	0.095		0.0050	0.050	0.0040
K		0.200	2.60	0.10			0.095	0.0050	0.050	0.0040
L			1.00	1.00			0.95	0.050	0.50	0.040

Table 2. Concentration of some elements in nutrient media. Media A-D served as reference media. The concentration of K was adjusted to 2.00 mM in media E, G, J (in medium A 1.95 mM) and to 0.200 mM in media F, H, K (in medium C 0.195 mM).

Medium	Reference medium with same concentration of elements			Approximate concentration, mM			
	Ca, Mg, Fe, P, S,	K	N	K	N	Na	Cl
A	A	A	A	2	3	0.1	-
G	A	A	C	2	0.3	0.1	3
H	A	C	A	0.2	3	2	-
L	A	-	A	-	3	2	-
B	B	B	B	0.6	1	0.03	-
E	B	A	C	2	0.3	0.03	2
F	B	C	A	0.2	3	2	-
C	C	C	C	0.2	0.3	0.01	-
J	C	A	C	2	0.3	0.01	2
K	C	C	A	0.2	3	3	-
D	D	D	D	0.06	0.1	0.003	-

Table 3. Effect of partial removal of roots of A-plants on number of transpiration peak during the first light period. Light intensity was 28-30 klux. Remaining roots of root-. plants were seminal.

Number of remaining roots	Number of experiments giving				Total number of experiments
	⩽ 2 peaks	3-5 peaks	> 5 peaks, damped oscillation	sustained oscillation	
5-7 (intact plants)	3	2	4	1	10
3	0	1	1	4	6
2	2	1	1	1	5

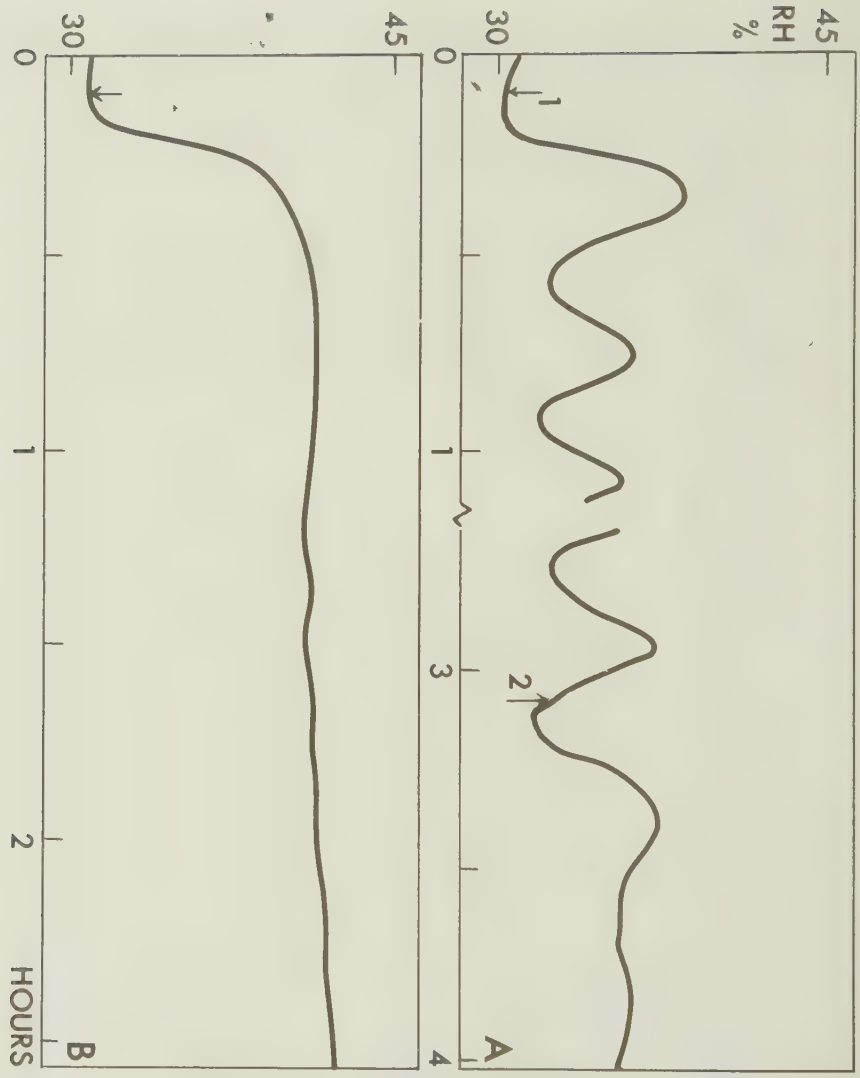

Figure 1. Different stomatal behaviour of plants from chamber II. Air flow rate 40 l/h, relative air humidity (RH) "30" %. Figure 1 A: 1 C-plant. Light at 1, light intensity 5 klux. Root excision under water at 2. Figure 1 B: 1 A-plant. Light at arrow, light intensity 27 klux.

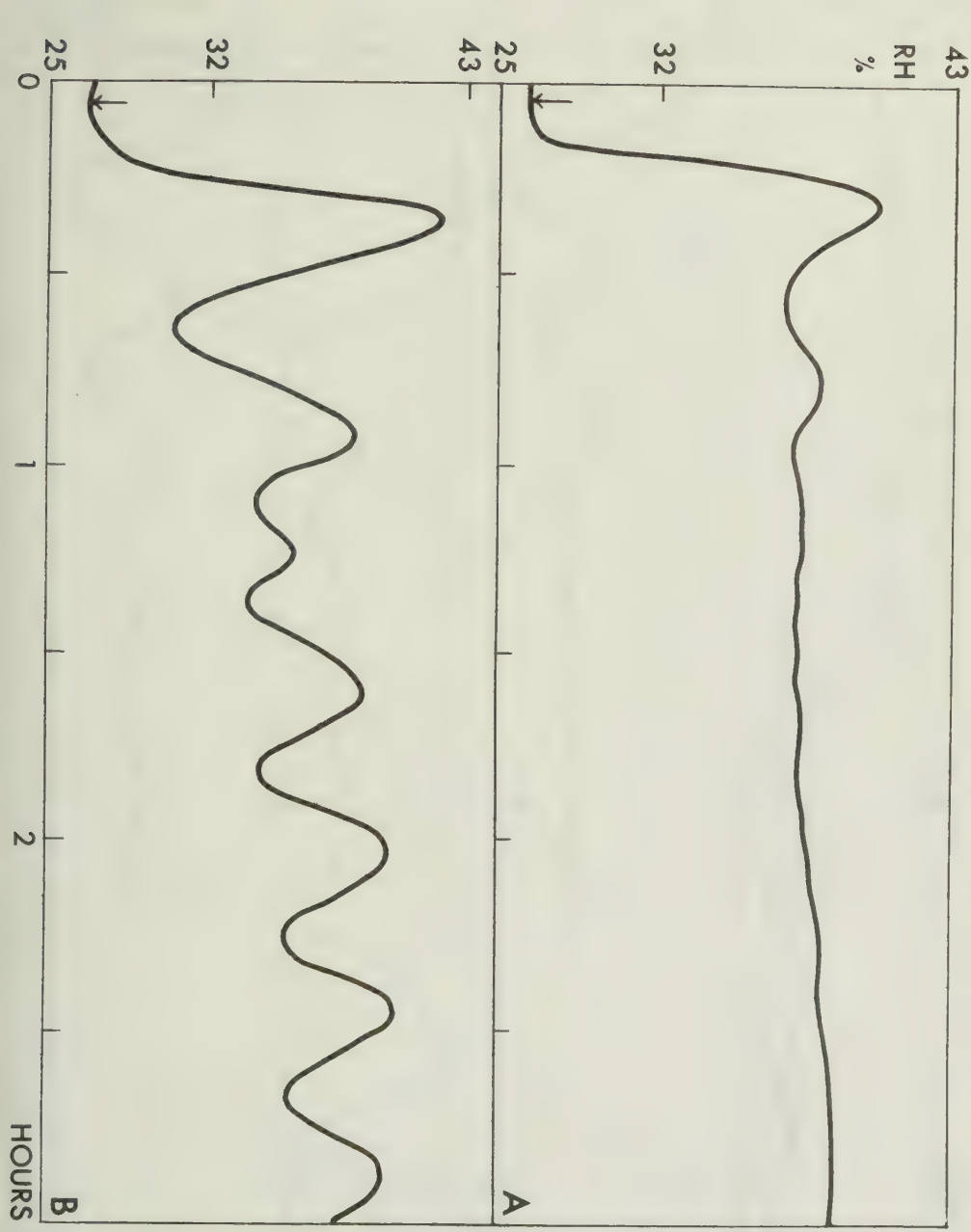

Figure 2. Effects of light pretreatment on transpiration of plants grown in a day-night light regime. 2 A-plants from chamber II in each experiment. The experiments were performed the same day. Air flow rate 100 l/h, air humidity (RH) 25 %. Light at arrow, light intensity 25 klux. Figure 2 A. Plants taken 1.25 h from the beginning of the light period. Figure 2 B. Plants taken 15.7 h from the beginning of the light period.

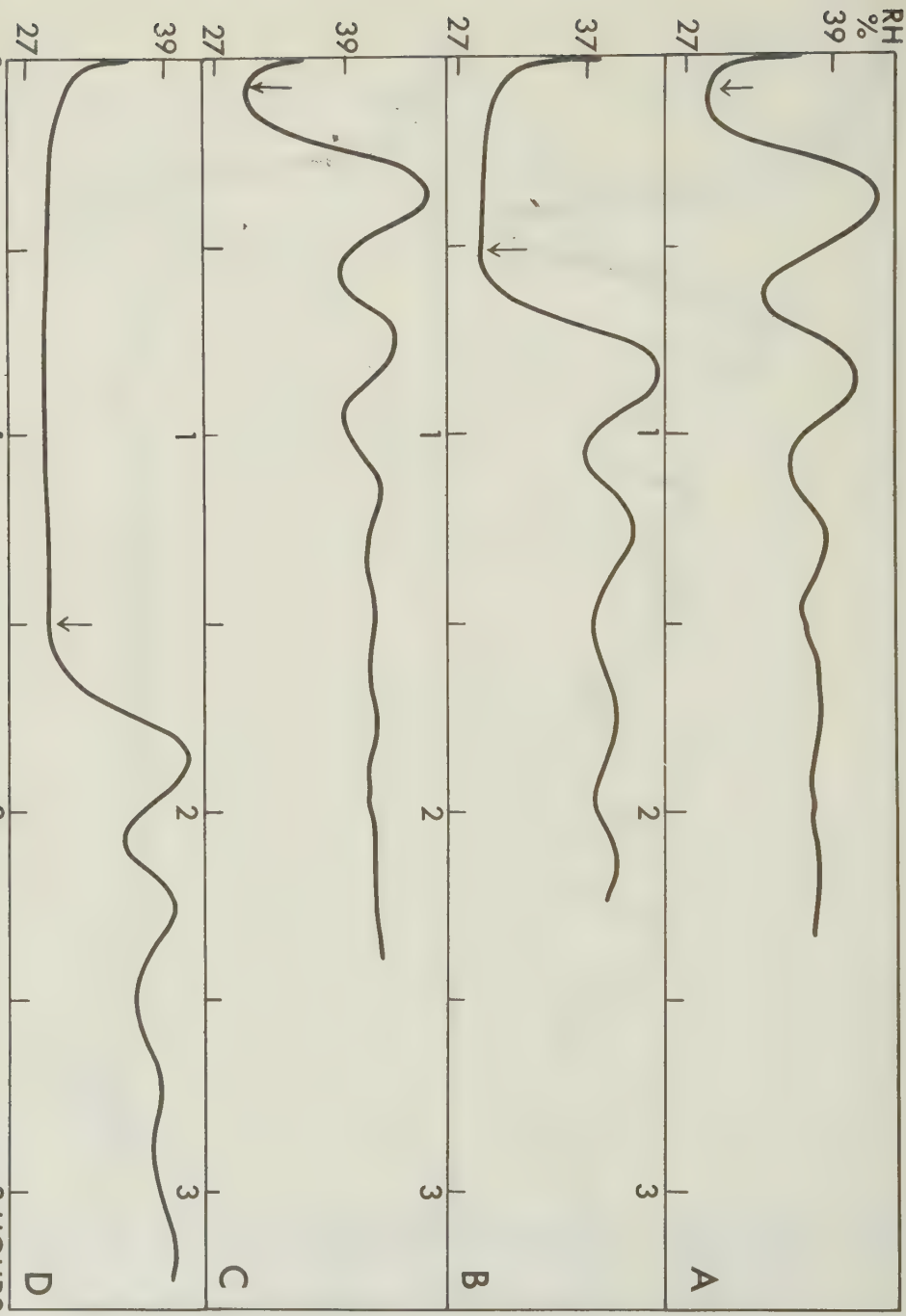

Figure 3. Effect of time of day and length of the initial dark period on transpiration of plants grown in continuous light. The experiments were run in the order A-D with about 8 h between the first and the last start. 3 A-plants from chamber I in each experiment. Air flow rate 100 l/h, air humidity (RH) 27 %. Light at arrow, light intensity 27 klux. Length of the initial dark period: A 5 min, B 30 min, C 5 min, D 90 min.

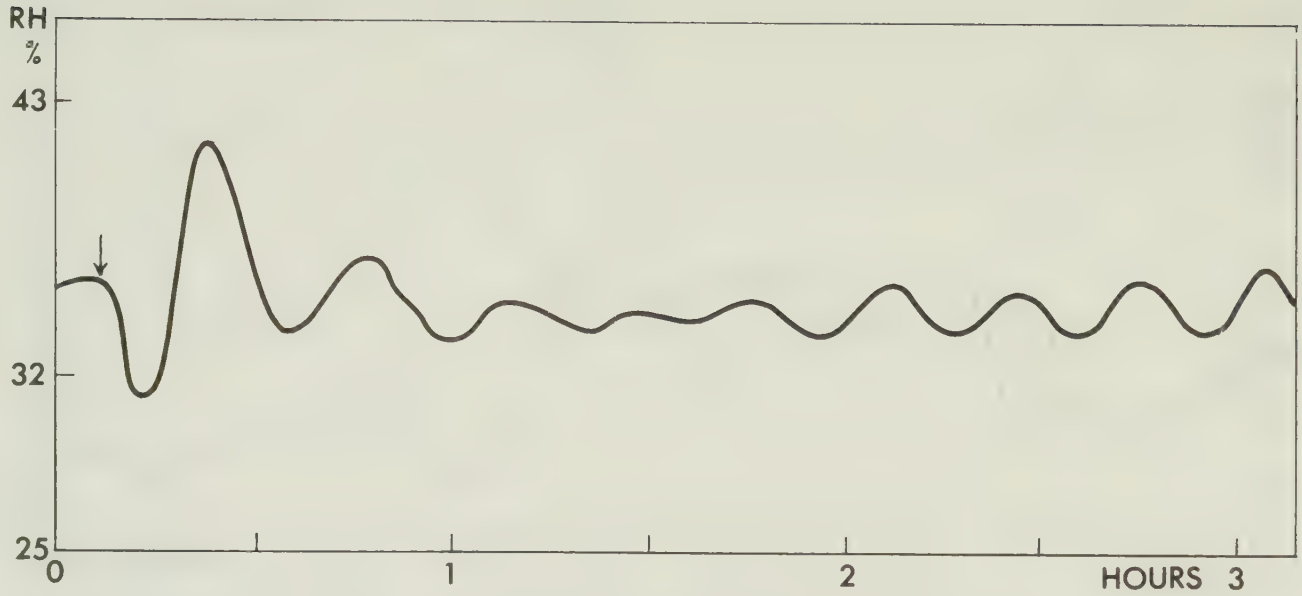

Figure 4. Induction of stomatal cycling with a dark pulse. 2 A-plants from chamber II. Air flow rate 100 l/h, air humidity (RH) 25 %. Light intensity 25 klux. A dark pulse with a duration of 1 min was given at arrow.

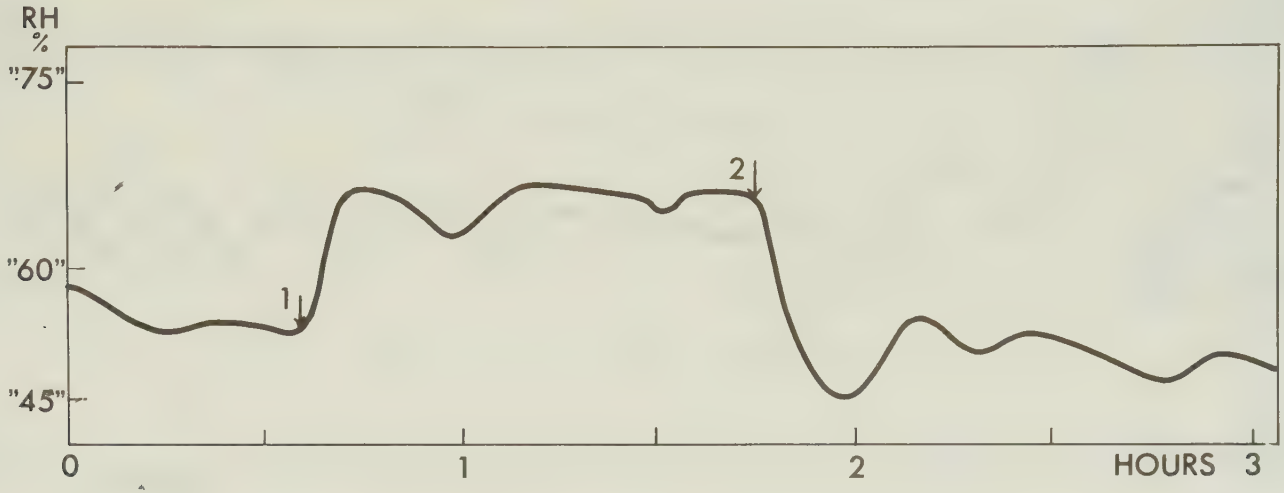

Figure 5. Effects of step changes of light intensity. 2 C-plants from chamber II. Air flow rate 20 l/h, air humidity (RH) "30" %. Light intensity 9.5 klux. 1. Light intensity 32 klux. 2. Light intensity 9.5 klux.

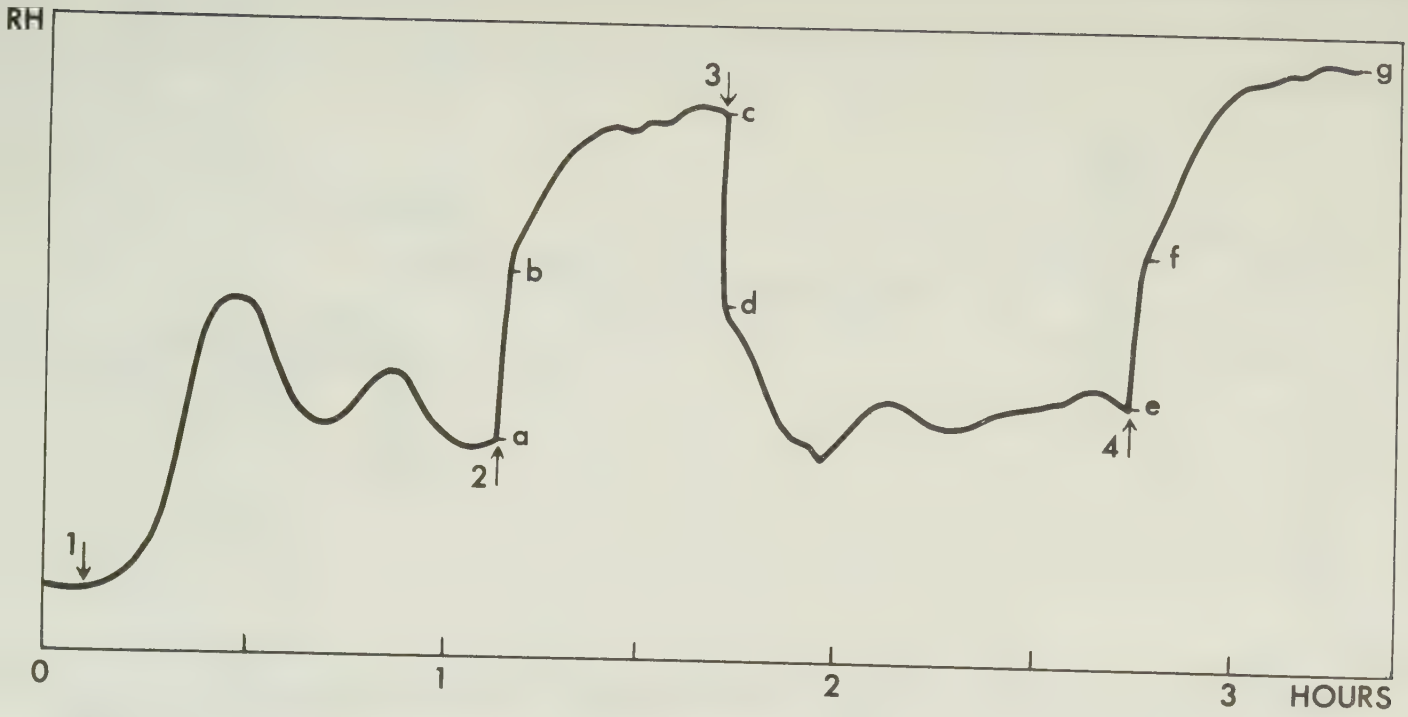

Figure 6. Effects of step changes of the air flow rate. 4 A-plants from chamber I. Air flow rate 59 l/h, air humidity (RH) 30 %. Light at 1, light intensity 21 klux. 2. Air flow rate 20 l/h. 3. Air flow rate 60 l/h. 4. Air flow rate 20 l/h.

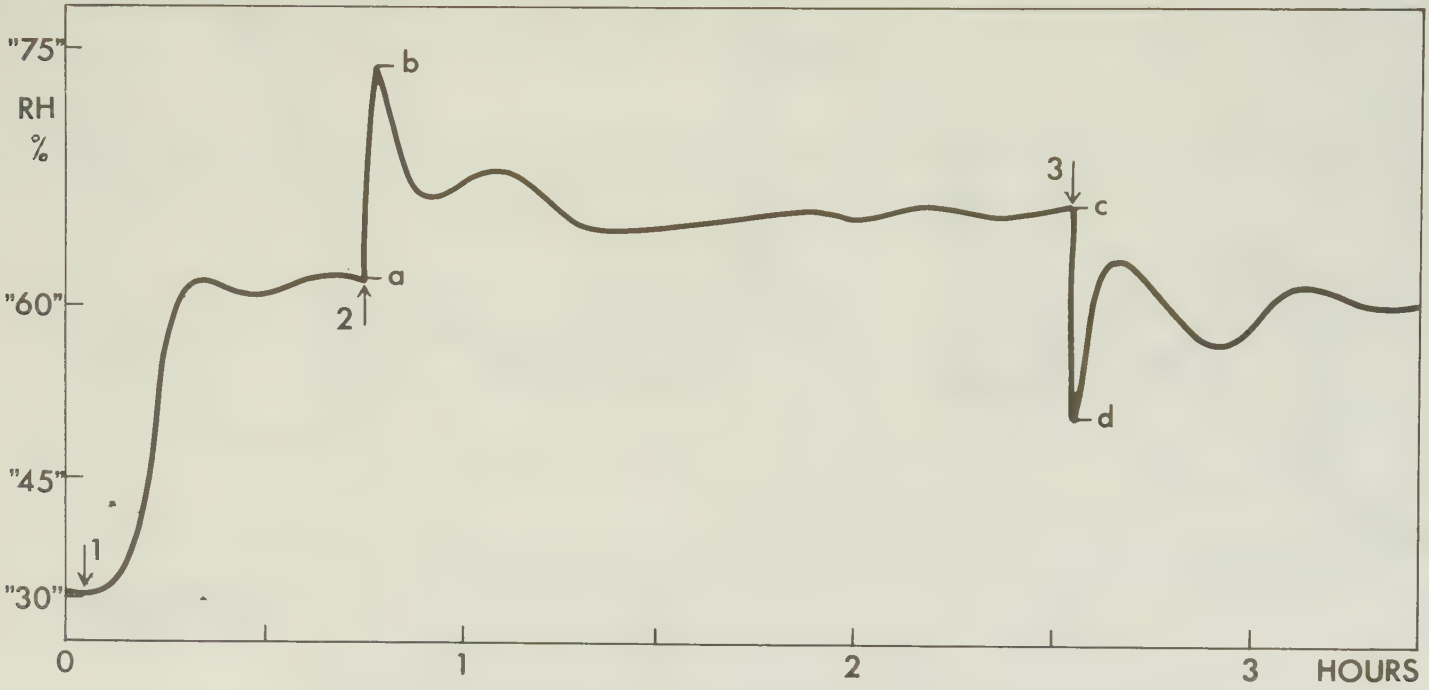

Figure 7. Effects of simultaneous step changes of air flow rate and light intensity. 5 B-plants from chamber II. Air flow rate 60 l/h, air humidity (RH) "30" %. Light at 1, light intensity 27 klux. 2. Air flow rate 20 l/h, light intensity 5.9 klux. 3. Air flow rate 60 l/h, light intensity 27 klux.

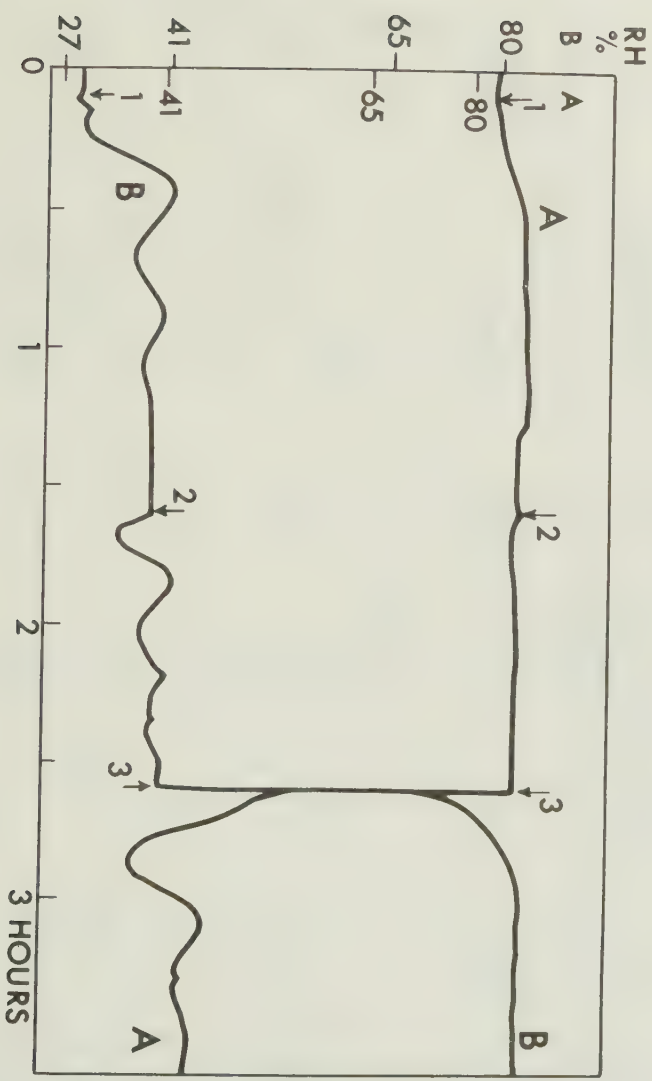

Figure 8. Effects of step changes of air humidity. 3 B-plants from chamber I in each experiment. Air flow rate 100 l/h. Light intensity 27 klux. Figure 8 A. Air humidity (RH) 80 %. 1. Light. 2. 1 min dark pulse. 3. Air humidity 27 %. Figure 8 B. Air humidity 27 %. 1. Light. 2. 1 min dark pulse. 3. Air humidity 80 %.

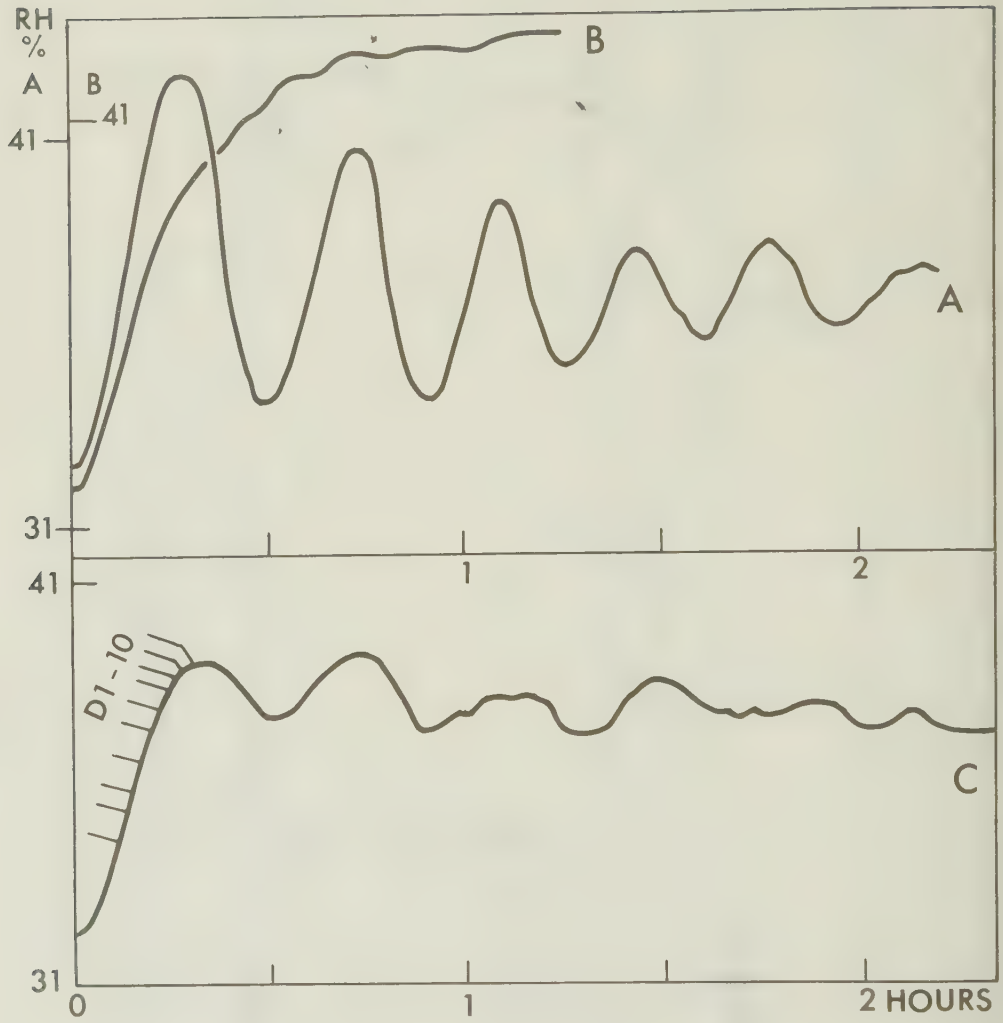

Figure 9. Effects of root removal under water in the dark and of dark pulses during stomatal opening. 2 E-plants from chamber I in each experiment. Air flow rate 100 l/h, air humidity (RH) 31 %. Light at time zero, light intensity 44 klux. Figure 9 A. Intact plants. Figure 9 B. Roots removed under water during the initial dark period. Figure 9 C. D 1-10. Dark pulses with durations between 5 and 30 sec.

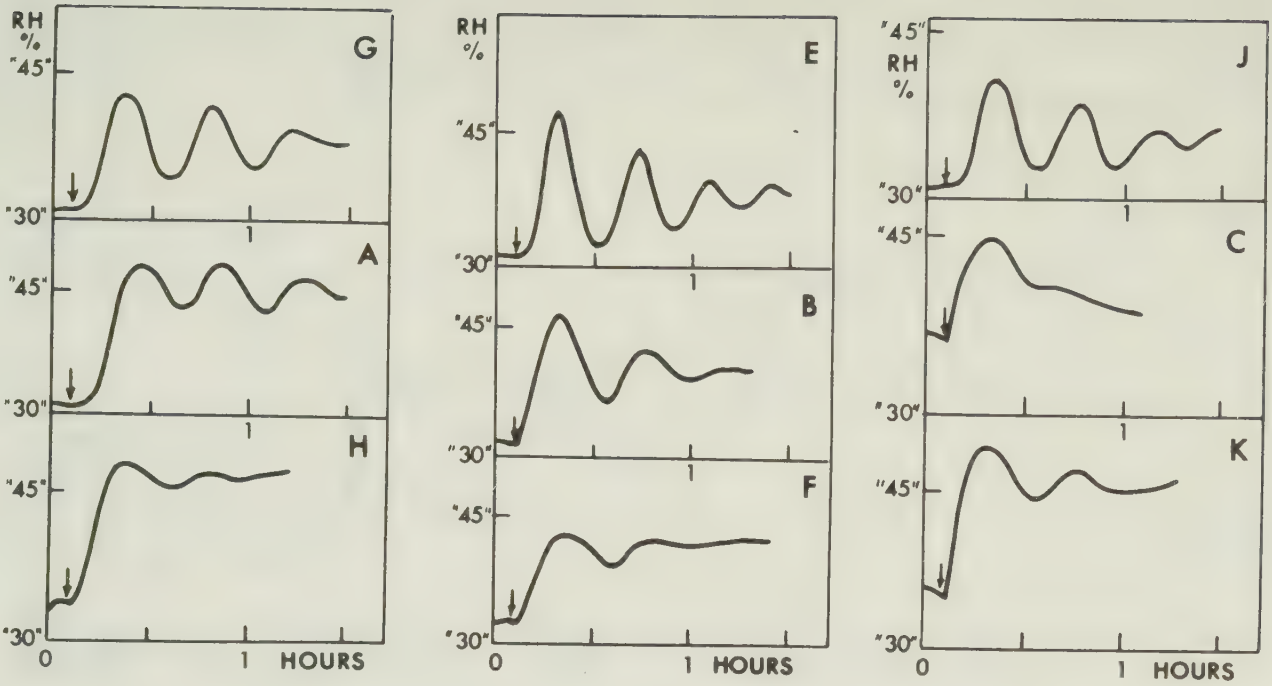

Figure 10. Effects of nutrient supply. The capitals indicate plants used. Number of plants: 3 G, 3 A, 3 H, 4 E, 3 B, 2 F, 4 J, 4 C, 3 K. Air flow rate 60 l/h, air humidity (RH) "30" %. Light at arrows, light intensity 26 klux (E,B,F) or 27 klux (remaining experiments).

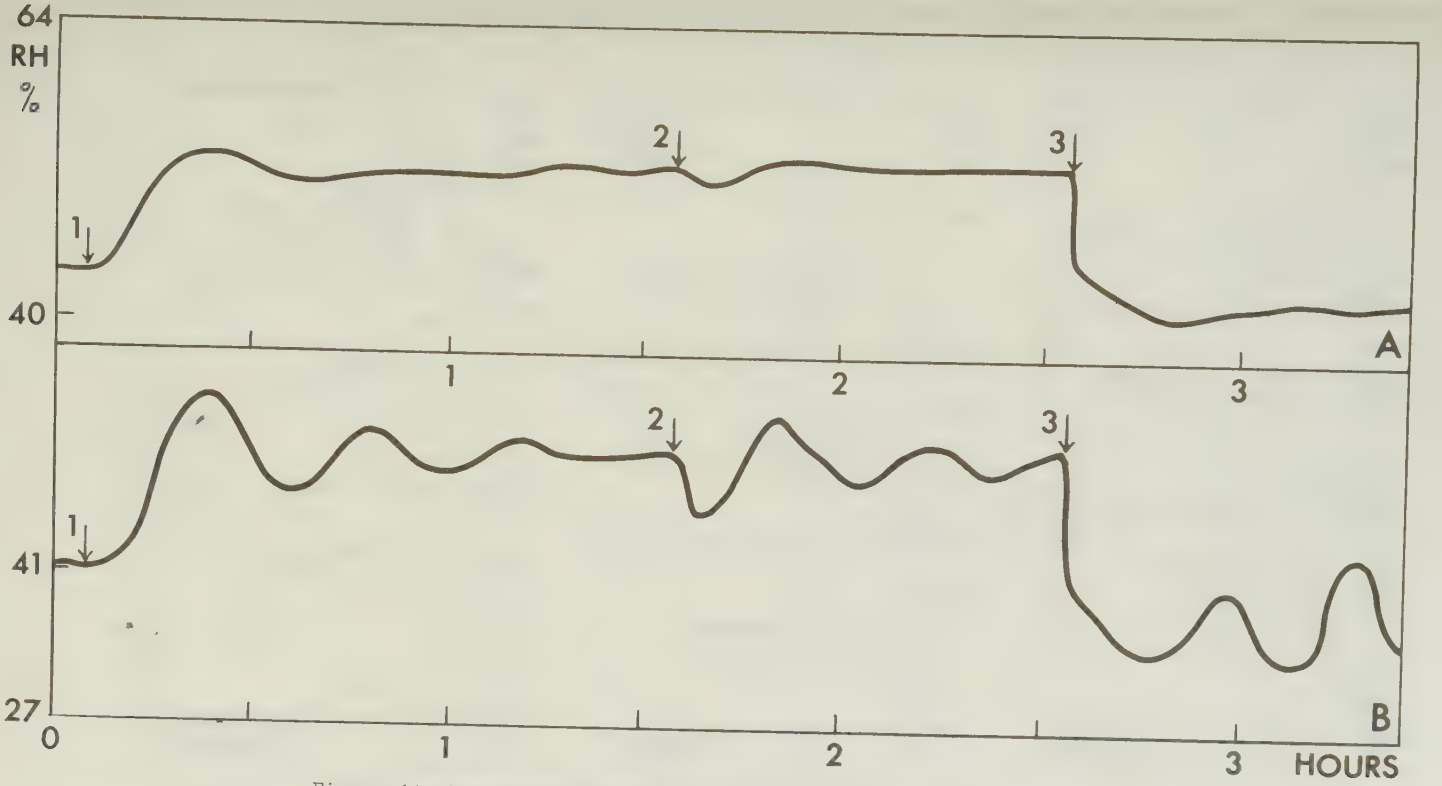

Figure 11. Comparison between cycling tendencies of F- and E-plants. 3 plants in each experiment. Air flow rate 100 l/h. Light intensity 27 klux. Figure 11 A. F-plants. Air humidity (RH) 40 %. 1. Light. 2. 1 min dark pulse. 3. Air humidity 26 %. Figure 11 B. E-plants. Air humidity 41 %. 1. Light. 2. 1 min dark pulse. 3. Air humidity 27 %.

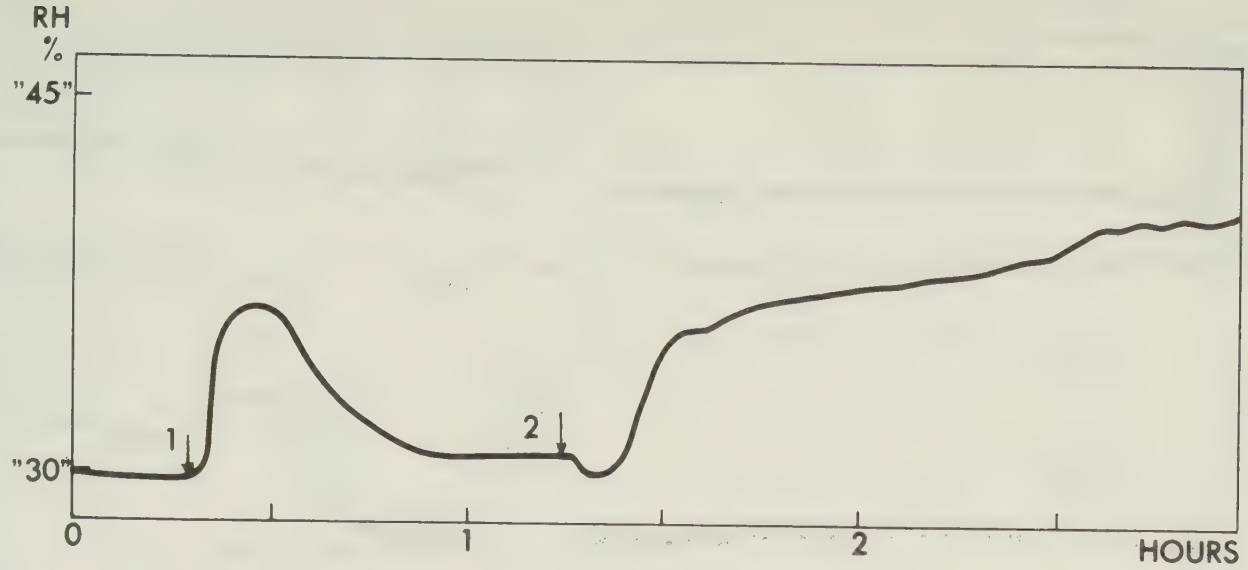

Figure 12. Effect on a C-plant from chamber II of illumination at a high light intensity. Air flow rate 40 l/h, air humidity (RH) "30" %. 1. Light at an intensity of >100 klux. 2. Roots cut under water.

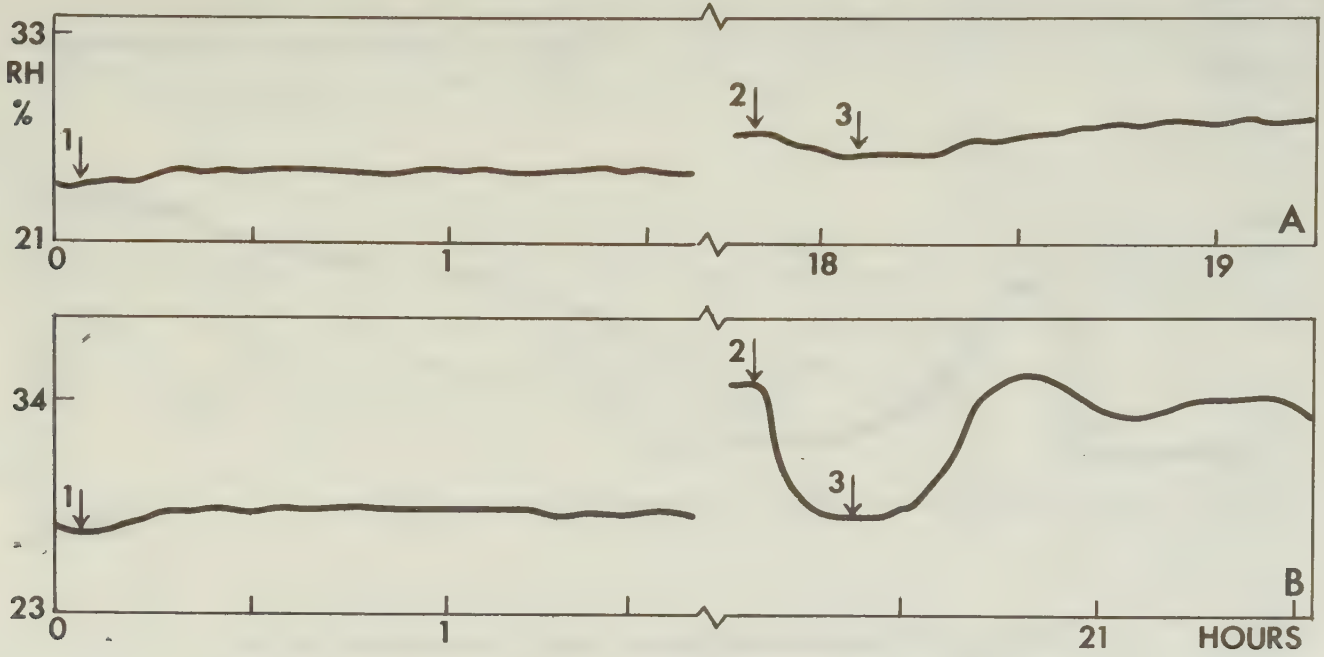

Figure 13. Effects of root treatment of L-plants with KCl. 4 plants from chamber I in each experiment. Air flow rate 100 l/h. Figure 13 A. Root medium distilled water. Air humidity (RH) 21 %. Light intensity 29 klux. 1. Light. 2. Dark. 3. Light. The calibration uncertain for the second part of the curve. Figure 13 B. Root medium 5 mM KCl. Air humidity 23 %. 1. Light. Light intensity 28 klux. 2. Dark. 3. Light.

Figure 14. Successive effects of root treatment of L-plants with KCl. 4 plants from chamber I. Root medium 2 mM KCl. Air flow rate 100 l/h, air humidity (RH) 23 %. Initial dark period 107 min. 1. Light at an intensity of 27 klux. 2. Dark. 3. Light. 4. Dark. 5. Light.

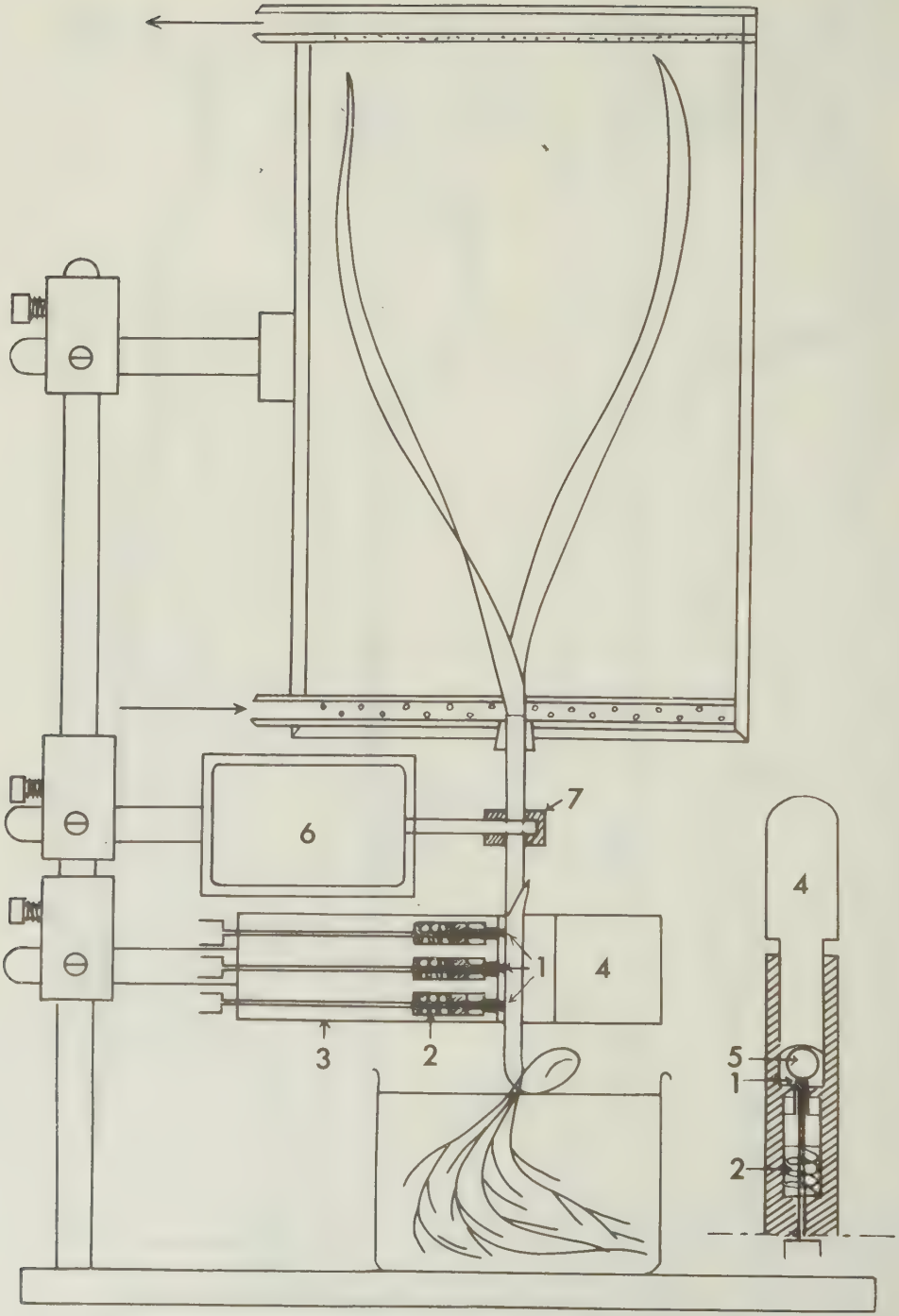

Figure 15. Experimental arrangement. 1. Thermistors. 2. Spring. 3. Perspex holder. 4. Perspex plate. 5. Stem. 6. Strain gauge transducer. 7. Support behind the stem.

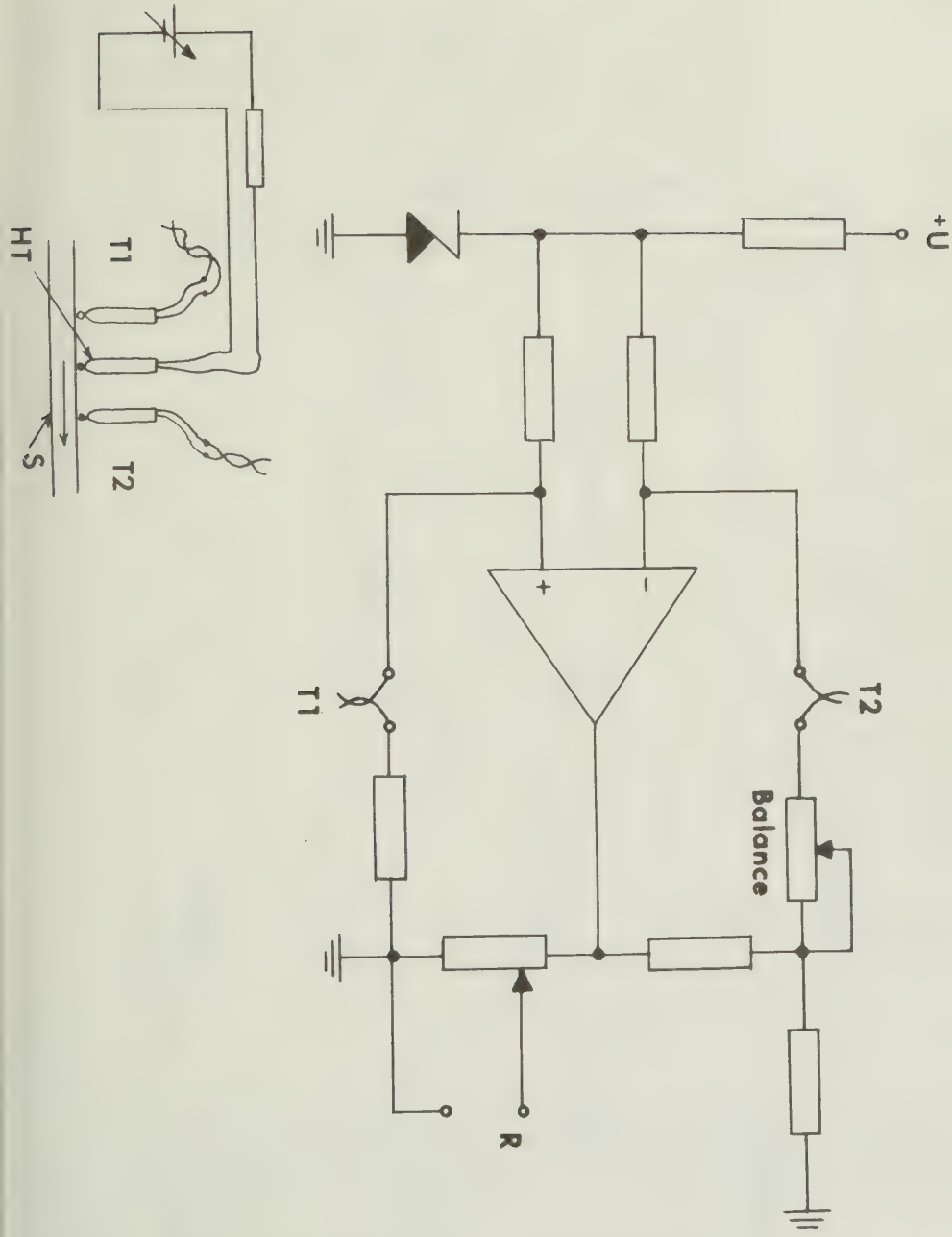

Figure 16. Scheme of flux meter. T1 and T2 thermistors coupled in a bridge. HT heating thermistor. S stem. R recorder.

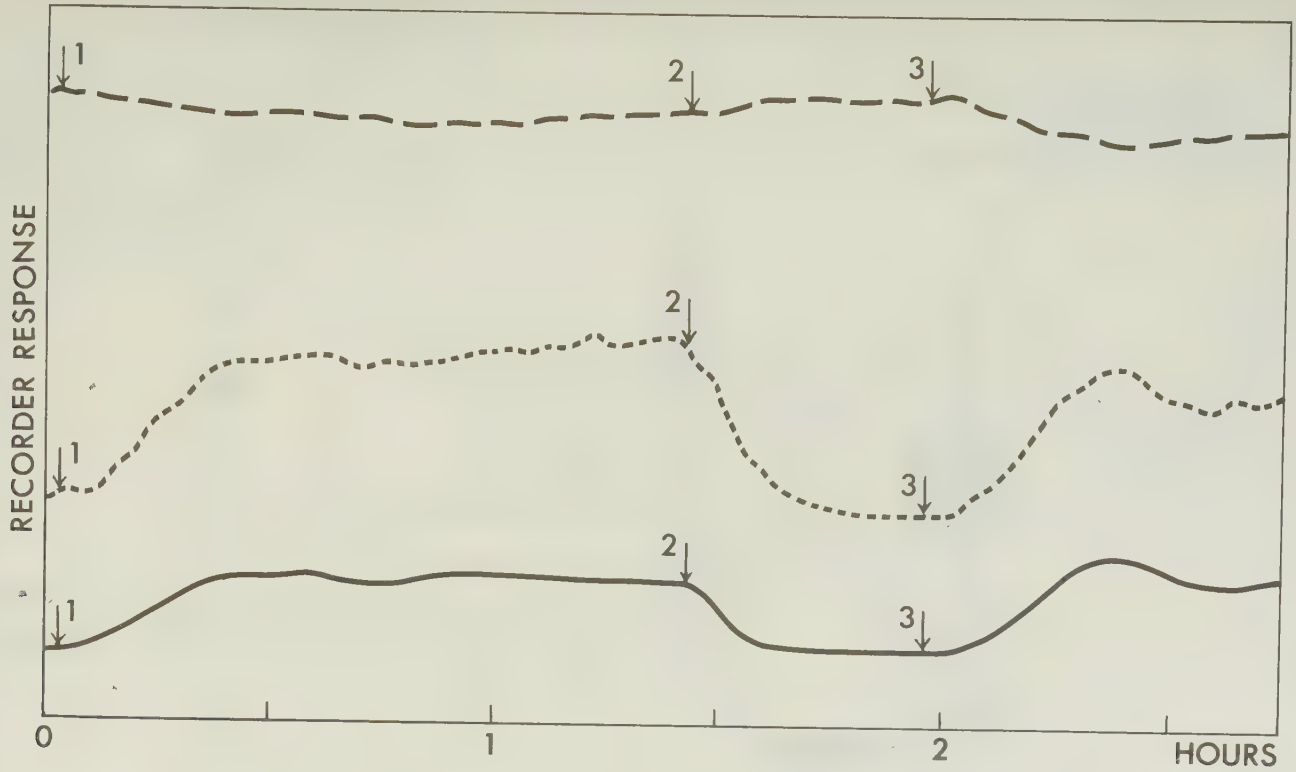

Figure 17. Time course of transpiration, sap flux, and stem diameter of a rootless plant. A-plant. Air flow rate 57 l/h, air humidity 33 %. Roots removed under water during the initial dark period. 1. Light at an intensity of 32 klux. 2. Dark. 3. Light. Transpiration ———— Sap flux ····· Stem diameter - - - -

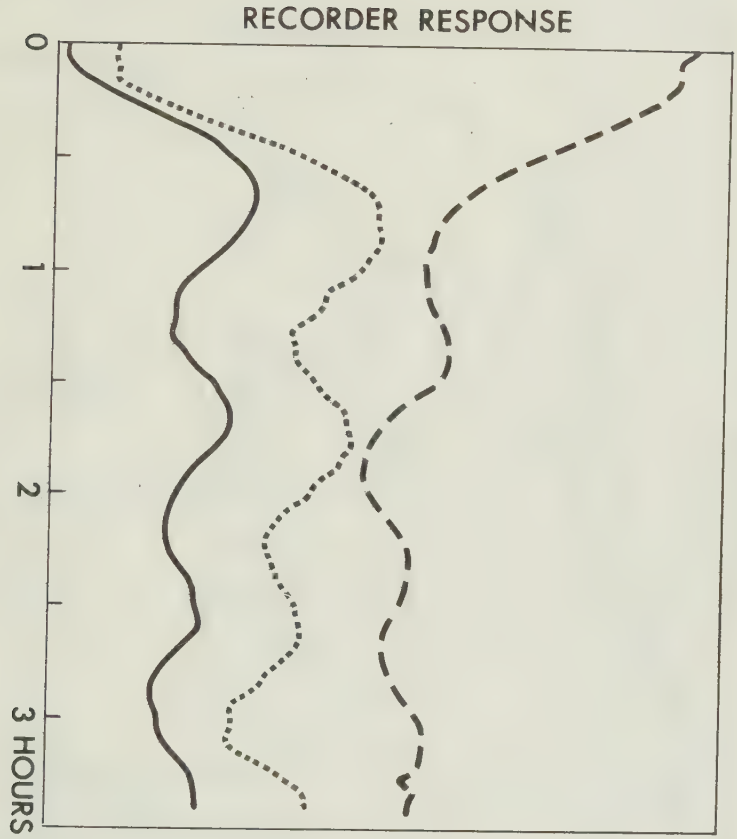

Figure 18. Phase relations between transpiration, sap flux, and stem diameter of an intact plant. A-plant. Air flow rate 56 l/h, air humidity 33 %. Light at time zero, light intensity 32 klux. Transpiration ———— Sap flux ····· Stem diameter - - - -

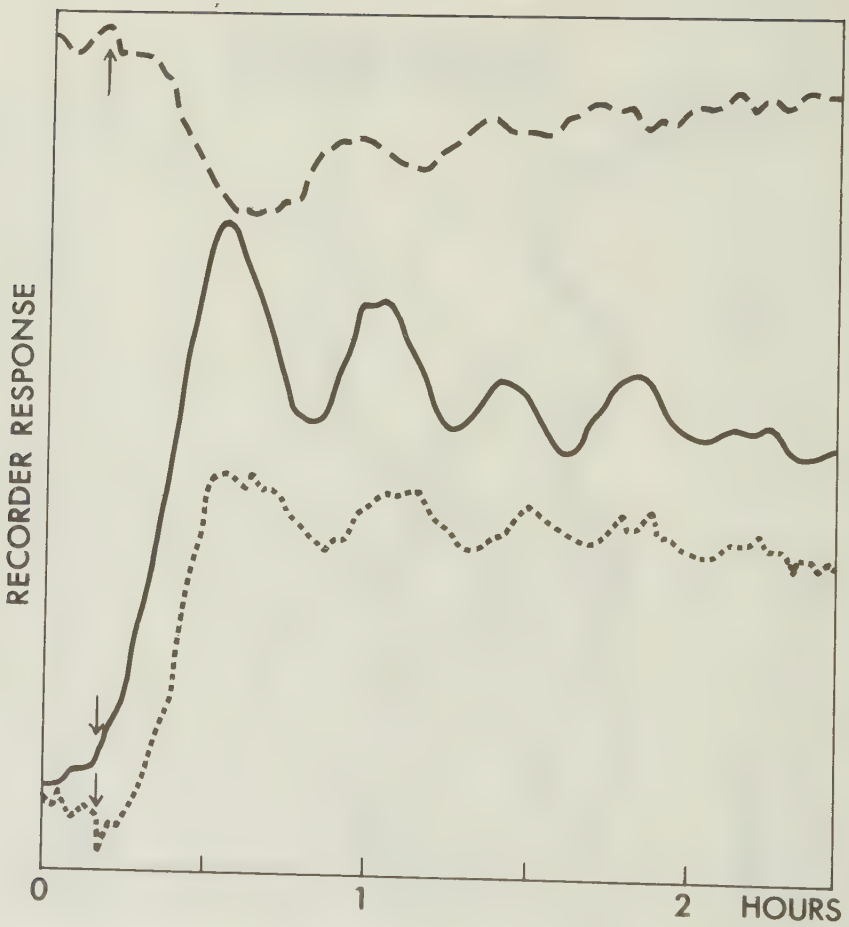

Figure 19. Damped oscillations in an intact plant. A-plant. Air flow rate 100 l/h, air humidity 10-12 %. Light at arrow at an intensity of 30 klux. Transpiration ———— Sap flux ····· Stem diameter - - - -

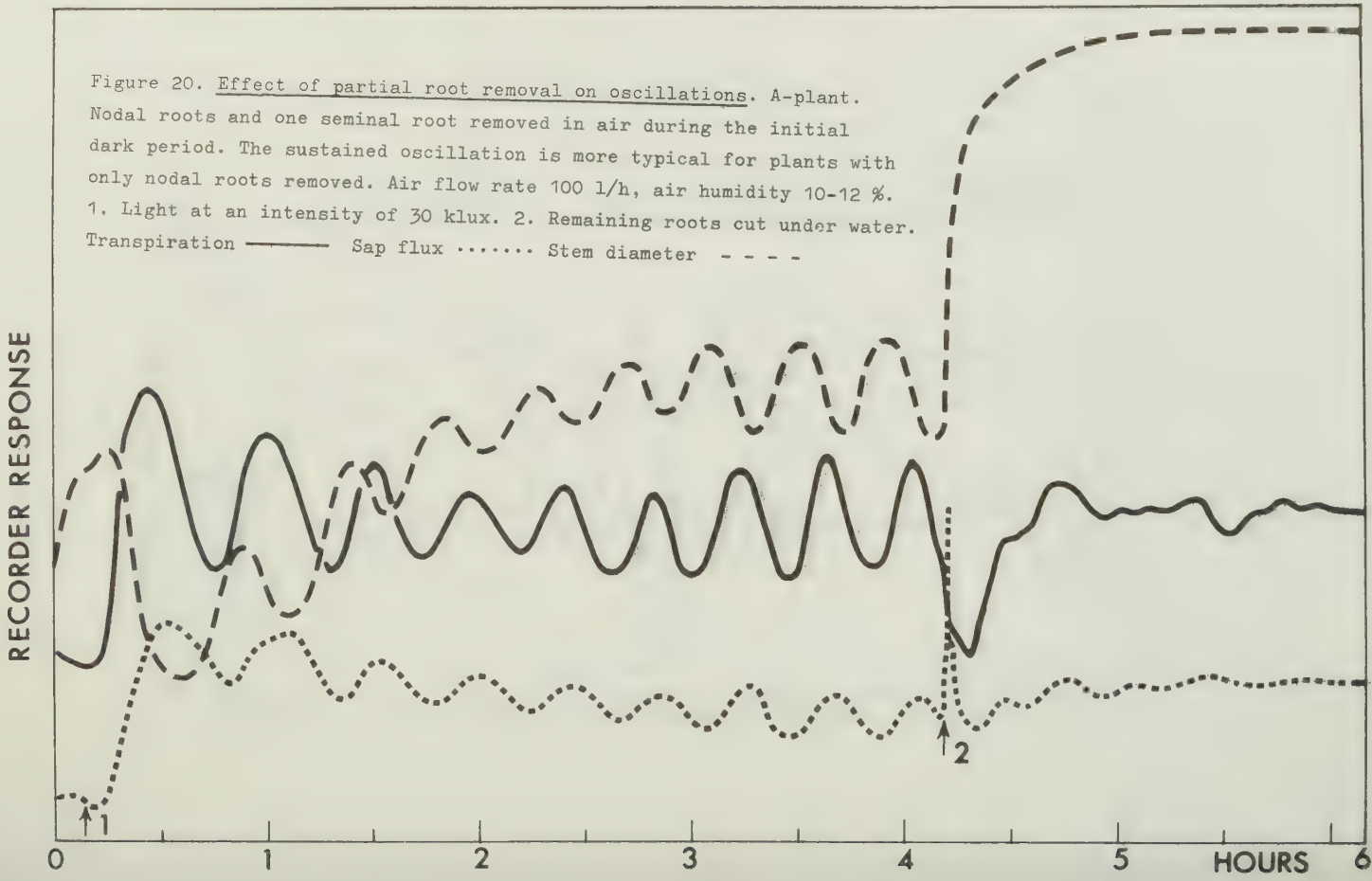

Figure 20. Effect of partial root removal on oscillations. A-plant. Nodal roots and one seminal root removed in air during the initial dark period. The sustained oscillation is more typical for plants with only nodal roots removed. Air flow rate 100 l/h, air humidity 10-12 %. 1. Light at an intensity of 30 klux. 2. Remaining roots cut under water. Transpiration ——— Sap flux ····· Stem diameter - - - -

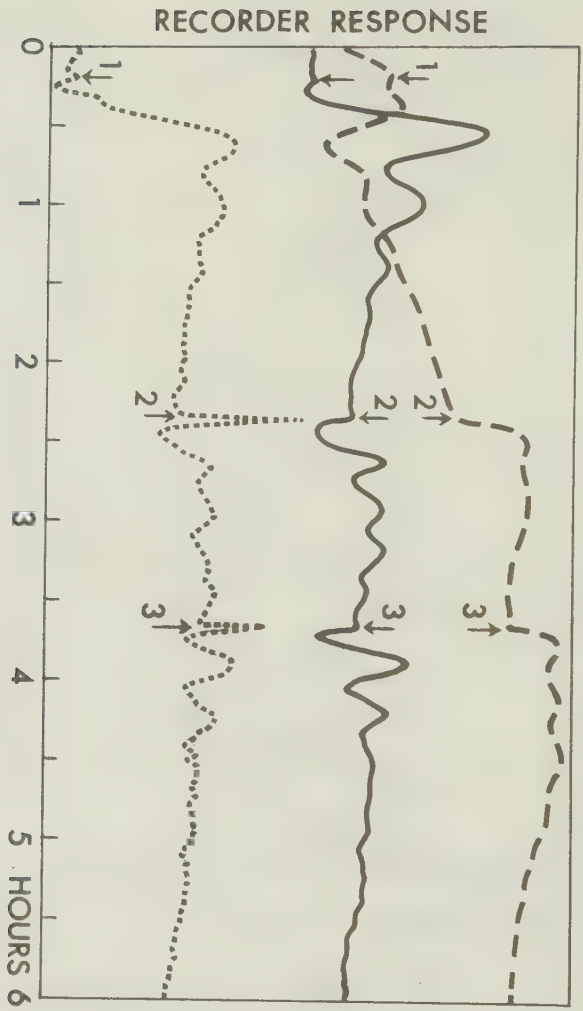

Figure 21. Effect of partial root removal on oscillations. A-plant. Nodal roots and one seminal root removed in air during the initial dark period, giving a typical result. Air flow rate 100 l/h, air humidity 18 %. 1. Light at an intensity of 29 klux. 2. One of two roots cut under water. 3. The second root cut under water. Transpiration ——— Sap flux ..... Stem diameter - - - -

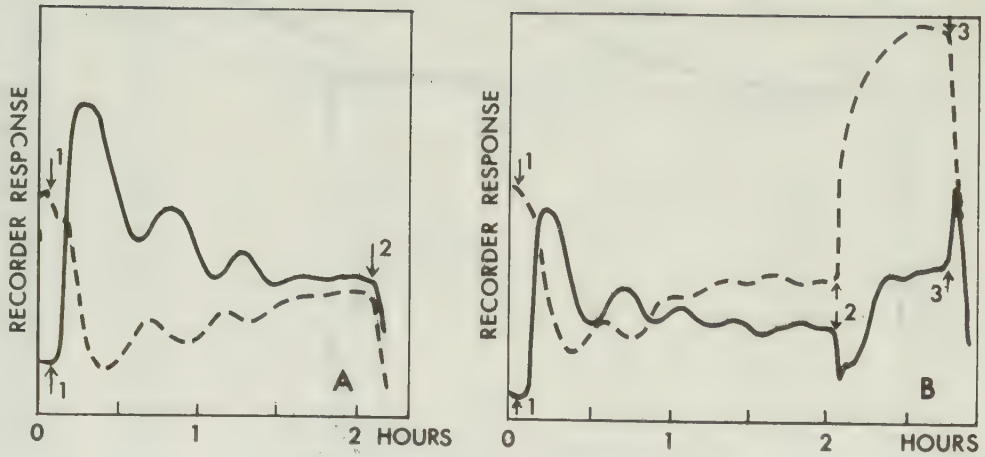

Figure 22. Effects of root cutting in air as influenced by a preceding root cutting under water. A-plant with only one remaining (seminal) root in each experiment. Air flow rate 100 l/h, air humidity 11 %. Light intensity 80 klux. Figure 22 A. 1. Light. 2. Root cut in air. Figure 22 B. 1. Light. 2. Root cut under water. 3. Root stump cut in air. Transpiration ———— Stem diameter - - - -

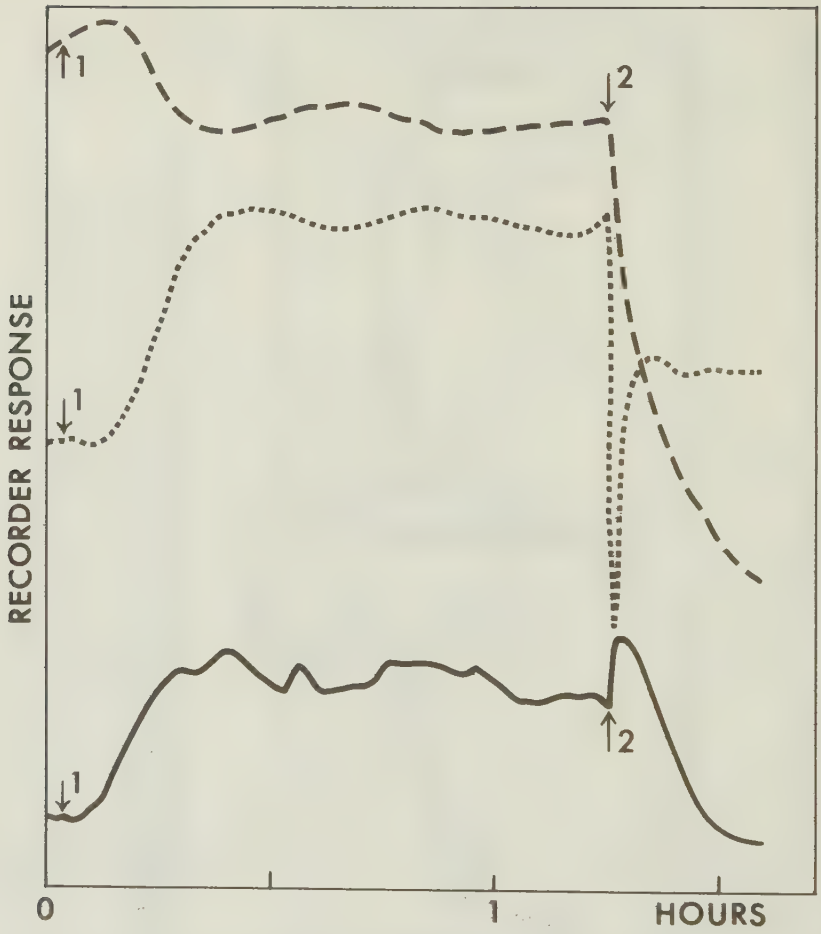

Figure 23. Effects of root treatment with NaCl. A-plant. Air flow rate 58 l/h, air humidity 33 %. 1. Light at an intensity of 32 klux. 2. Distilled water exchanged for about half concentrated NaCl. Transpiration ——— Sap flux ····· Stem diameter - - - -

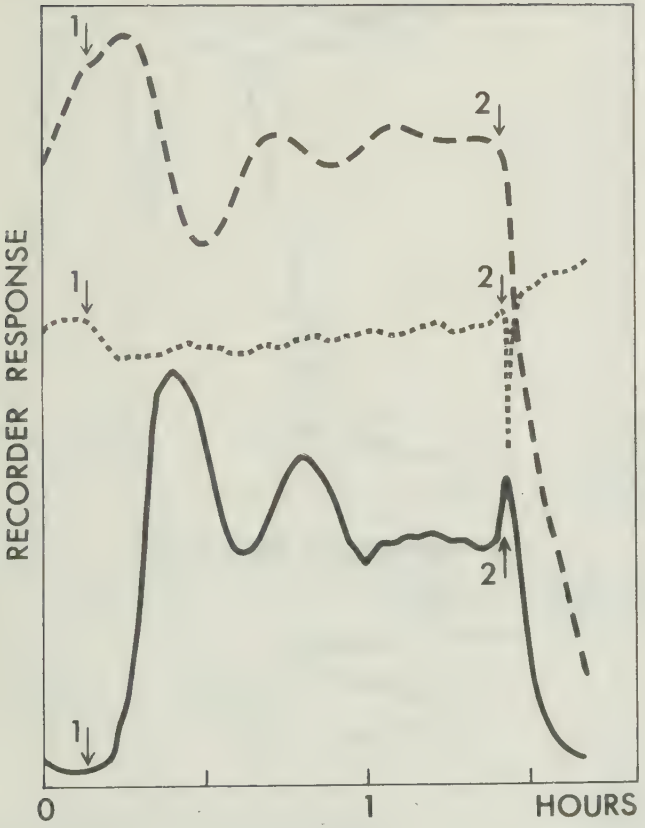

Figure 24. Effects of root treatment with NaCl. A-plant. Air flow rate 90 l/h, air humidity 24 %. Current to heating thermistor zero. 1. Light at an intensity of 25 klux. 2. Current to heating thermistor switched on and distilled water exchanged for concentrated NaCl. Transpiration ———— Sap flux ····· Stem diameter - - - -

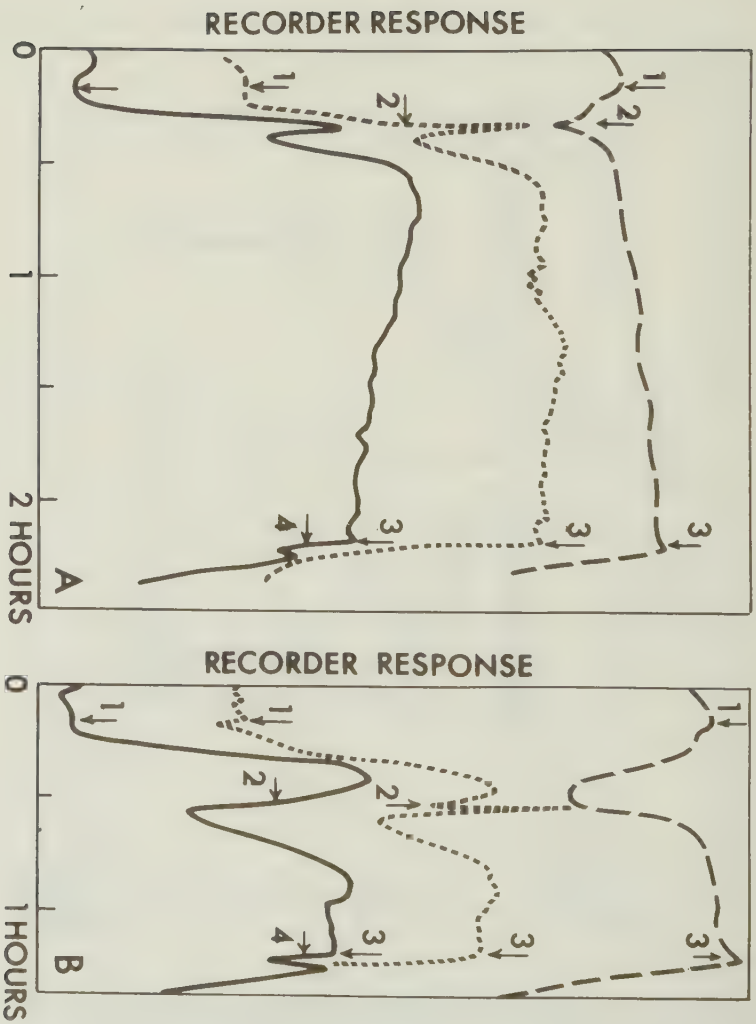

Figure 25. Effects on stomatal movements of root cutting under water and in air. A-plants. Air flow rate 100 l/h, air humidity 11 %. Figure 25 A. 1. Light at an intensity of 80 klux. 2. Roots cut under water. 3. Dark. 4. Root stumps cut in air. Figure 25 B. Light at an intensity of 86 klux. 2. Roots cut under water. 3. Dark. 4. Root stumps cut in air. Transpiration ——— Sap flux ····· Stem diameter - - - -

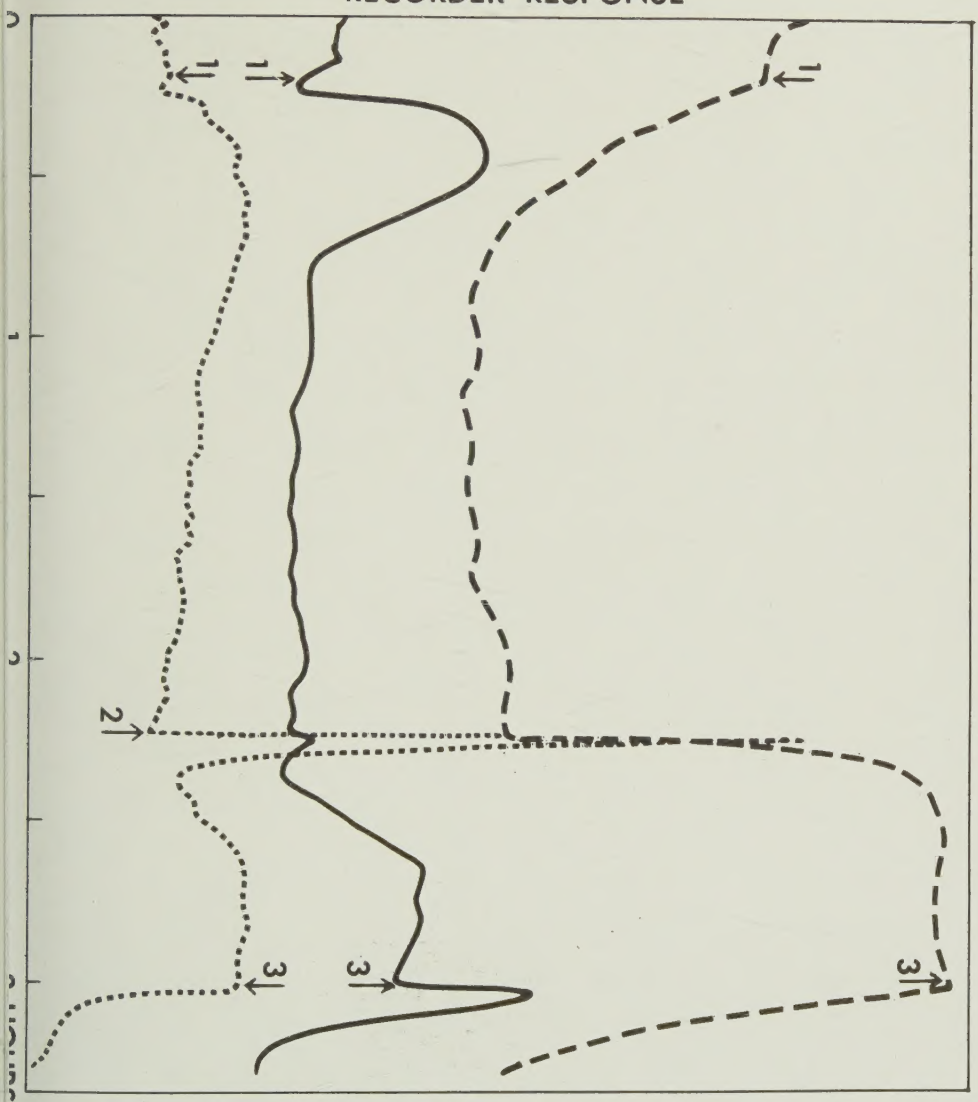

Figure 26. Effects of root removal under water during water stress. D-plant. All roots except one (seminal) removed in air during the initial dark period. Air flow rate 100 l/h, air humidity 11 %. 1. Light at an intensity of 82 klux. 2. The remaining root cut under water. 3. Root stump cut in air. Transpiration ——— Sap flux ····· Stem diameter - - - -

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